DOI:10.14753/SE.2023.2857

MOLECULAR BACKGROUND OF RESISTANCE TO TARGETED THERAPIES IN CHRONIC LYMPHOCYTIC LEUKEMIA

PhD THESIS

Lili Kotmayer, MD

Pathological Sciences Doctoral School

Semmelweis University

Supervisors: **Csaba Bödör, PhD, DSc**
Donát Alpár, PhD

Official reviewers: **Gergely Varga, MD, PhD**
Tibor Pankotai, PhD

Head of the Complex Examination Committee: Janina Kulka, MD, PhD, DSc

Members of the Complex Examination Committee: **András Masszi, MD, PhD**
Laura Horváth, MD, PhD

Budapest
2023

DOI:10.14753/SE.2023.2857

TABLE OF CONTENTS

DOI:10.14753/SE.2023.2857

DOI:10.14753/SE.2023.2857

LIST OF ABBREVIATIONS

Bcl-2	B cell lymphoma 2
BCR	B cell receptor
BTK	Bruton's tyrosine kinase
CI	confidence interval
CLL	chronic lymphocytic leukemia
CLL-IPI	CLL International Prognostic Index
CR	complete response
ddPCR	droplet digital polymerase chain reaction
del	deletion
ERIC	European Research Initiative on CLL
FA	fractional abundance
FC	fludarabine-cyclophosphamide combination therapy
FCR	fludarabine-cyclophosphamide-rituximab combination therapy
FDA	Food and Drug Administration
FISH	fluorescence *in situ* hybridization
IGHV	immunoglobulin heavy chain variable region
IGHV-B	borderline IGHV mutation status
IGHV-M	mutated IGHV mutation status
IGHV-U	unmutated IGHV mutation status
iwCLL	International Workshop on CLL
MIRA	Hungarian Ibrutinib Resistance Analysis Initiative
MRD	minimal residual disease
NGS	next-generation sequencing
ORR	overall response rate

DOI:10.14753/SE.2023.2857

OS	overall survival
PB	peripheral blood
PBMC	peripheral blood mononuclear cell
PFS	progression free survival
PI3K	phosphatidylinositol 3 kinase
R/R	relapsed or therapy refractory disease
trisomy 12	trisomy of chromosome 12
VAF	variant allele frequency

DOI:10.14753/SE.2023.2857

I. INTRODUCTION

Chronic lymphocytic leukemia (CLL), a malignant tumor of mature B cells, is one of the most common types of oncohematological diseases in the developed countries with an age-adjusted incidence of 4-5 per 100,000 people. CLL is characterized by the monoclonal expansion and proliferation of typically CD5 expressing and mature B cells in the peripheral blood, bone marrow and other lymphoid organs, including lymph nodes and the spleen. CLL is most common in the elderly, with a median age of 72 years at the diagnosis and is affecting more males than females (2:1). (1)

Only a handful of risk factors has been described for CLL to date including exposure to hepatitis C virus and common infections, exposure to herbicides and pesticides, and notably hereditary factors. (1) CLL has the strongest inherited predisposition among hematologic malignancies. Although familial CLL is an extremely rare entity, about 10% of patients developing CLL has a family history of the disease, with the risk of CLL increasing to 7.5-8.5-fold in cases with affected first-degree relatives. (2)

CLL has highly variable and heterogeneous clinical manifestations, albeit the most common presentation of the disease is the incidental discovery of asymptomatic lymphocytosis during complete blood count analysis obtained for reasons unrelated to CLL. Lymphadenopathy is the second most common presentation, whereas the B symptoms, such as fever, weight loss, night sweats, and organomegaly present less frequently. Single- or multi-lineage cytopenias due to lymphocytic infiltration of the bone marrow may also be associated with the disease in some cases. (3)

Although CLL might be primary suspected in patients with the above-described clinical presentations, the diagnosis of CLL requires the sustained presence of $>5\times10^9$/L lymphocytes in the peripheral blood (PB). Based on the criteria of the International Workshop on CLL (iwCLL) working group, clonality of the B cells must be assessed by flow cytometry, demonstrating immunoglobulin light chain (Kappa or Lambda) restriction. (4) CLL cells typically co-express surface antigens CD5, CD19, CD20, and CD23 confirming the use of the panel of CD19/CD5/CD20/CD23/Kappa/Lambda immunophenotypic markers to establish the diagnosis recommended by the European Research Initiative on CLL (ERIC) and the European Society for Clinical Cell Analysis working groups. In ambiguous cases, the use of additional markers including CD38,

CD43, CD79b, CD81, CD200, CD10 and ROR1 may further contribute to the refinement of the diagnosis as well as distinguishing different prognostic subgroups. (5, 6)

I.1. Cytogenetic and molecular alterations with prognostic or predictive value in CLL

Monoclonal proliferation and expansion underlying CLL are characterized by the acquisition of distinct cytogenetic and molecular events leading to heterogeneous genetic, epigenetic and translational changes in patients with CLL and rendering the leukemia more aggressive. Approximately 80% of all cases carry at least one of the four most common chromosomal alterations with prognostic features detected by fluorescence *in situ* hybridization (FISH): deletions (del) of chromosomal regions 11q, 13q, 17p and trisomy of chromosome 12 (trisomy 12). (7)

Isolated del(17p) found in 5-8% of chemotherapy-naïve cases frequently includes the tumour suppressor gene *TP53* and similar to pathogenic *TP53* mutations, is a strong predictive biomarker for resistance against conventional chemotherapies. (8) Notably, up to 80% of the patients with confirmed del(17p) also harbour *TP53* mutations in the remaining allele. (9) Del(13q) is the most common chromosomal aberration detected in up to 55% of the cases and is characterized by favourable outcomes. (10) Del(11q) encompassing the gene *ATM* was associated with bulky disease and shorter overall survival rates, although its prognostic value has been overcome by the introduction of highly efficient cytoreductive chemoimmunotherapies. (7, 8, 11) Trisomy 12 is observed in 10-20% of the cases and is associated with intermediate prognosis. Further aberrations, such as deletion of 6q, trisomy 3 and translocation t(14q32), are additional findings in CLL patients and do not influence the overall survival (OS), however they may contribute to complex karyotype in some cases. (7) Complex karyotype, defined by the presence of ≥5 chromosomal abnormalities, discriminates poor outcome and is an independent prognostic biomarker for response to chemoimmunotherapeutic agents, but its use does not extend to the standard-of-care diagnostics and is mostly limited to clinical trials and other studies (Table 1). (7, 10, 12, 13)

In addition to cytogenetic changes, recent studies utilizing whole exome sequencing to characterize the genetic landscape of CLL have uncovered a number of recurrently

mutated genes involved in DNA damage signalling and repair, RNA processing, MAPK signalling and MYC activity. These genes include *TP53, ATM, NOTCH1, BIRC3, SF3B1, MYD88, RPS15, FBXW7, POT1, CHD2, IKZF3, ZNF292, ARID1A, ZMYM3* and *PTPN11*. (10, 14-16) Mutations of *TP53* associated with dismal prognosis are identified in 4-37% of the cases and have a detrimental effect on response to chemotherapy. Predictive value of *TP53* disruptions encompassing del(17p) and *TP53* mutations was confirmed by several research groups as well as our own dataset (*unpublished observations*). (9, 17) Pathogenic variants of other recurrently mutated genes are most likely not of prognostic or predictive significance.

Table 1. Independent prognostic and predictive biomarkers in chronic lymphocytic leukemia

Biomarker	Prognosis	Method	Indication
del(11)q	unfavourable	FISH	prior to every line of therapy
del(13q)	favourable	FISH	prior to every line of therapy
del(17p)	unfavourable	FISH	prior to every line of therapy
trisomy 12	intermediate	FISH	prior to every line of therapy
complex karyotype	unfavourable	karyotyping	clinical studies only
IGHV-U	unfavourable	Sanger-sequencing or NGS	at the time of progression
TP53 mutations	predictive	Sanger-sequencing or NGS	at the time of progression

del = deletion; FISH = fluorescence in situ hybridization; IGHV-U = unmutated heavy chain of the B cell receptor immunoglobulin; NGS = next-generation sequencing

Somatic hypermutation of the heavy chain of the B cell receptor immunoglobulin (IGHV) is an additional widely used and independent prognostic biomarker in CLL with unmutated IGHV status (IGHV-U, ≥98% germline homology) conferring aggressive illness and shorter OS, while those carrying mutated IGHV (IGHV-M, <97% germline homology) have a more indolent clinical course. (6, 18, 19) Borderline hypermutation load with germline homology of 97-98% represents a subgroup of CLL patients with intermediate prognosis (IGHV-B). Notably, up to 30% of the cases carry quasi-identical IGHV, a phenomenon aptly named B cell receptor stereotypy, underlying the putative

role of antigen selection in the pathogenesis of CLL. (20, 21) Among the numerous subsets associated with a spectrum of co-occurring gene mutations and variable clinical courses, stereotyped B cell receptor subset #2 is particularly noteworthy, since it confers dismal outcome regardless of the IGHV somatic hypermutation status. (21, 22)

Recently, epigenetic changes and permissive environment has also emerged as putative driver or co-operating events of leukemogenesis, but their role in guiding clinical patient care remains unclear to date.

I.2. Risk stratification and indication for treatment initiation

Substantial heterogeneity of the genetic background and initial presentation of CLL is complemented by a highly variable clinical course. Some patients survive for relatively long periods without requiring therapeutic intervention, whereas others succumb to an aggressive and rapidly progressing illness that is often refractory to standard chemoimmunotherapies. Patients with established diagnosis of the disease should undergo clinical staging and symptom-based risk stratification to assess the necessity for treatment initiation and expected prognosis. Classical clinical staging systems developed by Rai (23) and Binet (24) relying on physical examination findings and standard-of-care laboratory tests are – along with symptomatic or active disease – the basis of treatment indication and prognostication in both clinical practice and clinical trials, and do not involve imaging, or cytogenetic and molecular data.

Recent advances in molecular biology and understanding of the genetic changes driving leukemogenesis have led to the identification of markers associated with high-risk disease and progression conferring poor prognosis. In addition, the plethora of potential biomarkers identified are used independently of the clinical staging systems to provide prognostic information and further distinguish prognostic subgroups. Currently, one of the most relevant and comprehensive prognostic score incorporating clinical, biological and genetic data is the CLL International Prognostic Index (CLL-IPI) using five distinct markers for prognosis prediction, including age, clinical stage, serum β2-microglobulin, *TP53* dysfunction (deletion of 17p and *TP53* mutations) and unmutated IGHV status. (25, 26) Although the prognostic value of CLL-IPI is currently being revised for the

application of targeted therapies, it is a widely used tool for prognostication by separating four patient subgroups with significantly different 5-year survival rates (Figure 1).

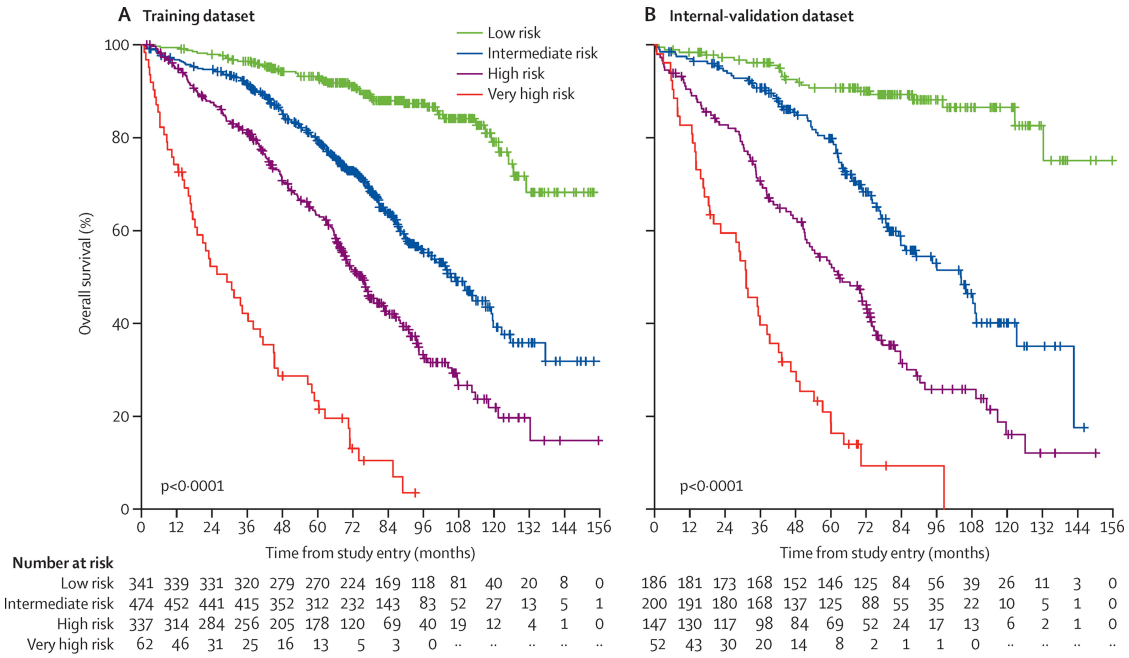


Figure 1. Overall survival according to the CLL-IPI risk groups based on (A) the training set of 1214 patients and (B) the internal validation dataset of 585 cases. Adapted from The International CLL-IPI working group. (26)

Criteria for treatment initiation proposed by the iwCLL working group are active or symptomatic disease and rapid progression, whereas patients with asymptomatic early stage (Rai 0-II, Binet A or B) disease do not benefit from early therapeutic intervention and should only be monitored for substantial signs of progression ('watch and wait strategy'). (4, 27) In cases with progressive, symptomatic or active disease based on defined criteria by the iwCLL working group (Table 2), therapy should be initiated with chemoimmunotherapeutic or targeted agents.

Table 2. Criteria to define active disease according to the iwCLL guidelines (4)

1.	Evidence of progressive bone marrow failure manifested as the development or worsening of anemia and/or thrombocytopenia
2.	Massive (i.e. ≥6 cm below the left costal margin) or progressive or symptomatic splenomegaly
3.	Massive nodes (i.e. ≥10 cm in longest diameter) or progressive or symptomatic lymphadenopathy

4. Progressive lymphocytosis with an increase of ≥50% over a 2-month period, or lymphocyte doubling time <6 months. Factors contributing to lymphocytosis other than CLL (eg, infections, steroid administration) should be excluded.
5. Autoimmune complications including anemia or thrombocytopenia poorly responsive to corticosteroids
6. Symptomatic or functional extranodal involvement (eg, skin, kidney, lung, spine)
7. Disease-related symptoms as defined by any of the following:
   a) Unintentional weight loss ≥10% within the previous 6 months
   b) Significant fatigue (i.e. Eastern Cooperative Oncology Group performance scale 2 or worse; cannot work or unable to perform usual activities).
   c) Fevers ≥38.0°C for 2 or more weeks without evidence of infection
   d) Night sweats for ≥1 month without evidence of infection

I.3. Therapeutic landscape of CLL

I.3.1. Chemotherapeutic and chemoimmunotherapeutic modalities

First systematic treatment option for CLL introduced in the 1960s was the use of steroids and the first-in-class alkylating agent chlorambucil applied in both front-line and relapsed CLL. Chlorambucil-based therapy was followed by the introduction of another alkylating agent cyclophosphamide and the nucleoside analogue fludarabine laying the foundations of combined chemotherapeutic protocols in CLL. Fludarabine-cyclophosphamide (FC) combination therapy was the first major breakthrough in the treatment of the disease achieving complete response (CR) rates and overall response rates (ORR) of 24% and 94%, respectively, and has become the first treatment of choice in front-line setting since 2007. (28) Introduction of the anti-CD20 antibody rituximab in 2005 brought the era of chemoimmunotherapy in CLL via the combined application of rituximab with the FC regimen (FCR). FCR was the first chemotherapy-based regimen to induce and sustain durable CR and achieve improved OS rates compared to FC. Today chemoimmunotherapy is mostly reserved for the front-line treatment of young and fit CLL patients carrying mutated IGHV without *TP53* disruption. (10)

I.3.2. Novel targeted agents in CLL

Clinical management of cases with relapsed or therapy refractory (R/R) CLL or treatment-naïve disease with high-risk genetic features as well as older and unfit CLL

DOI:10.14753/SE.2023.2857

patients has been revolutionized by the introduction of novel targeted agents. Over the last decade, a handful of potent targeted drugs with various targets have been introduced to clinical practice (Figure 2).

I.3.3. B cell receptor signalling pathway inhibitors

B cell receptor (BCR) signalling pathway activation supports CLL cell survival, and its continuous or repetitive signalling is pivotal in leukemogenesis implying the specific inhibition of the BCR signalling pathway as an encouraging strategy for the treatment of CLL. (29) Targeting the tyrosine kinase members of BCR signalling, particularly the Bruton's tyrosine kinase (BTK) and the phosphatidylinositol 3 kinase (PI3K), has substantially improved the therapeutic landscape of B cell malignancies and led to remarkable response rates in high-risk CLL patient populations.

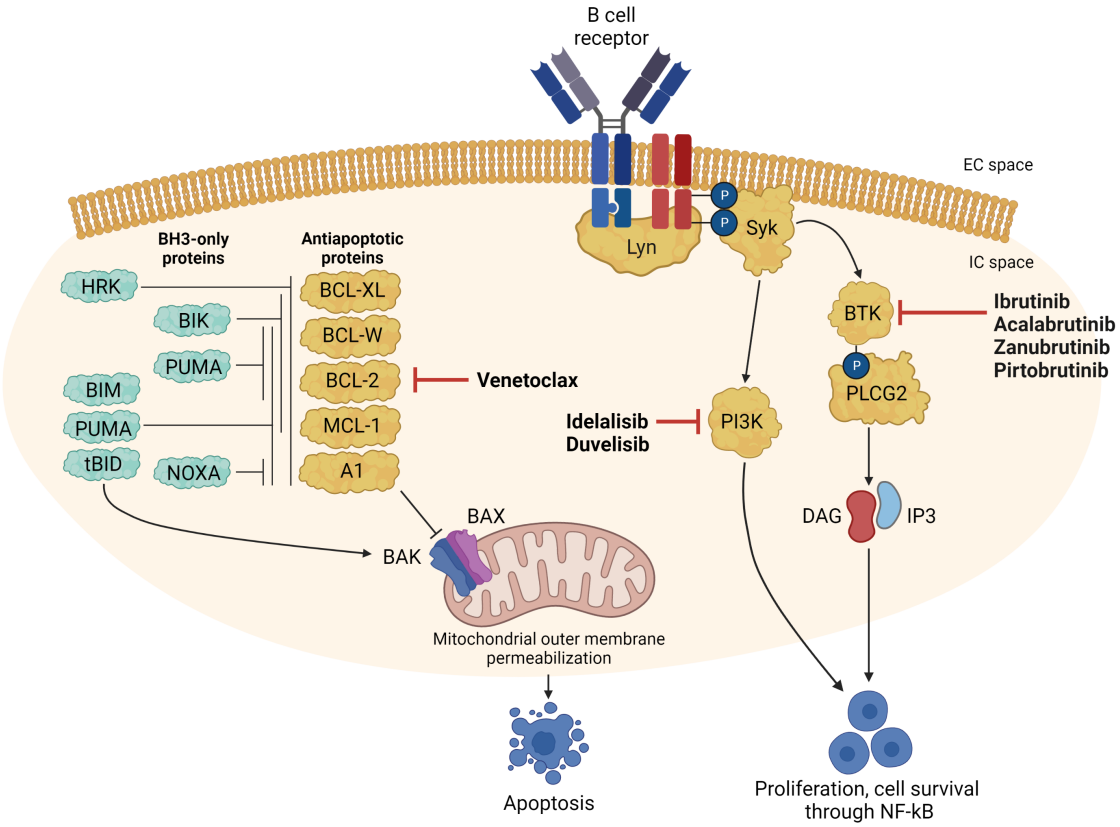


Figure 2. Targeted agents and their respective intracellular targets in CLL. Recently, the irreversible Bruton's tyrosine kinase (BTK) inhibitors ibrutinib, acalabrutinib and zanubrutinib together with the Bcl-2 inhibitor venetoclax have entered clinical practice achieving remarkable response rates across heterogeneous patient populations. The reversible Btk inhibitor pirtobrutinib, and the PI3K inhibitors idelalisib and duvelisib also confer durable remissions, however their use in standard patient care is limited in Hungary.

I.3.3.A. Phosphatidylinositol 3 kinase inhibitors

Currently, two novel agents targeting PI3K, namely idelalisib and duvelisib, have been approved by the Food and Drug Administration (FDA) in R/R settings. Based on the results of a phase 1 clinical trial, specific targeting of the constitutively activated PI3Kδ in patients with extensive prior therapy by first-in-class agent idelalisib *plus* rituximab combination resulted in an ORR of 72% with a median progression free survival (PFS) of 15.8 months. (30) Single agent dual inhibitor of PI3Kδ and γ duvelisib showed an ORR of 56% in a phase 1 trial involving 55 R/R CLL patients and is approved for the treatment of CLL after at least two prior lines of therapy. (31) Despite the remarkable response rates and notably the beneficial effect of idelalisib in cases with complex karyotype, today the use of PI3K inhibitors is limited to the treatment of R/R CLL patients with comorbidities, with other BCR antagonist tyrosine kinase inhibitors extending to clinical practice in Hungary. (32)

I.3.3.B. Inhibition of the Bruton's tyrosine kinase in CLL

Irreversible inhibitors of the Btk protein have essentially changed the treatment of patients with high risk CLL by inducing apoptosis in B cell malignancies through the disruption of downstream activation of the BCR pathway. This widely used class of targeted drugs, particularly ibrutinib, binding covalently to Btk are very potent therapeutic agents and together with Bcl-2 inhibition have become the main pillars of targeted therapy in CLL.

The first-in-class small molecule Btk inhibitor ibrutinib confers remarkable response rates in both treatment-naïve and R/R settings. (33-37) In one of the first pivotal trials with ibrutinib involving 85 patients with R/R CLL and small lymphocytic leukemia by Byrd and colleagues, ORR proved to be 71% in the heavily pre-treated patient population. (35) At 26 months of follow up, PFS and OS rates were 75% and 83%, respectively. An important finding of this study was that the response to ibrutinib seemed not to be influenced by clinical and genetic risk factors, such as advanced disease stage, number of prior therapy lines or the presence of del(17p), suggesting that ibrutinib achieves efficient disease control in both R/R and treatment-naïve high risk CLL. Since then, multiple phase 1-3 clinical trials (such as the pivotal RESONATE and RESONATE-2 studies) and real-world cohort analyses have shown the survival advantage conferred by ibrutinib over standard therapies, although one study by Thompson and colleagues reported inferior

outcome in R/R CLL patients with complex karyotype treated with ibrutinib-based regimens. (38-43)

Long-term follow up of patients treated with ibrutinib unveiled a distinct toxicity pattern putatively associated with multi-kinase inhibition and off-target effects. Most common side effects in ibrutinib-treated patient populations are cardiovascular abnormalities, including arrythmias, particularly atrial fibrillation requiring therapeutic anticoagulation, cardiac failure and bleeding. (10) Overall, despite the moderate toxicities, continuous Btk inhibition by ibrutinib is today widely used in clinical practice in both R/R cases and treatment-naïve patients carrying high-risk genetic features, such as *TP53* dysfunction and unmutated IGHV.

Recently, second generation, more selective irreversible BTK inhibitors, such as acalabrutinib and zanubrutinib were introduced to patient care in order to improve the efficacy and safety of BTK inhibition. Acalabrutinib was approved by the FDA in 2019 based on the results of the phase 3 clinical trials ELEVATE-TN and ASCEND involving 535 previously untreated and 310 R/R CLL patients, respectively. (44, 45) Median PFS and median OS were not reached in the acalabrutinib arms of either study, suggesting acalabrutinib as a potent novel agent in Btk inhibition. Zanubrutinib also demonstrated higher specificity and milder off target effect compared to ibrutinib in a phase 1 trial involving multiple B cell malignancies and was approved by the FDA in 2022 based on the results of the phase 3 clinical trials SEQUOIA and ALPINE in both R/R and front-line settings. (46-48) Although these novel agents together with the reversible BTK inhibitor pirtobrutinib (49) might address the unmet need for patients with intolerance to conventional BTK inhibition by ibrutinib, their use is mostly limited to clinical trials and has not yet been extended to standard-of-care clinical practice. (10)

I.3.4.Molecular background of resistance to ibrutinib

Despite the high response rates and prolonged PFS with ibrutinib, eventual loss of efficacy and subsequent Richter's transformation or acquired ibrutinib resistance occur in some patients, alike with other targeted therapies in hematologic and solid tumors. While Richter's transformation is recognized to occur within the first two years of ibrutinib therapy and its cumulative incidence plateaus after the third year, secondary or acquired ibrutinib resistance and subsequent progressive CLL emerges after longer

periods of targeted treatment (Figure 3). (50-54) Progressive CLL typically occurs two to five years after therapy initiation and is the leading cause of treatment failure. (52) Since secondary ibrutinib resistance confers dismal outcomes, early detection of loss of efficacy could potentially allow for the introduction of alternative treatment strategies with ideal timing over the disease course and the identification of patients who may benefit from change of therapy.

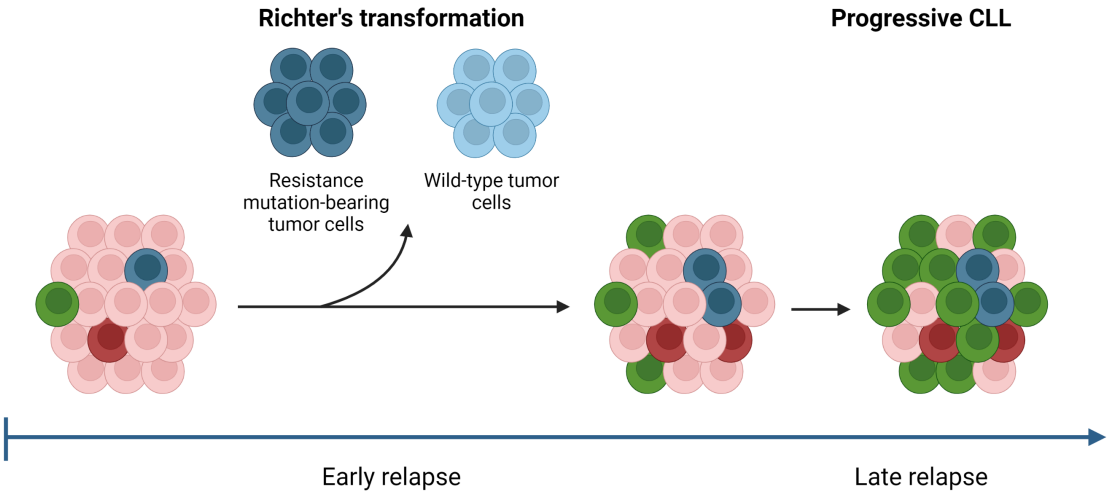


Figure 3. (Sub)clonal evolution of malignant cells in chronic lymphocytic leukemia (CLL). Over the disease course, proportion of CLL cells harbouring distinct subclonal genetic changes (green, red, and blue cells), present in small compartments in the original tumor cell population (pink cells), increases due to the positive selection pressure created by the different therapeutic agents. Following subclonal expansion underlying late relapse, the tumor's genetic profile differs significantly from its pre-treatment state.

Progressive CLL on ibrutinib is strongly associated with acquired (somatic) mutations in the ibrutinib binding site of BTK and/or the phospholipase Cγ2 (PLCG2) protein directly downstream of BTK in the BCR signalling pathway. Although additional del(8p) has also been linked to ibrutinib-relapsed cases by Burger and colleagues, *BTK* and *PLCG2* mutations are observed in the vast majority (up to 100%) of the patients experiencing progressive CLL on ibrutinib. (53, 55-57) *BTK* p.Cys481Ser missense mutation located at the ibrutinib binding site is by far the most common alteration associated with acquired ibrutinib resistance. The alteration diminishes the covalent binding between the protein and ibrutinib, thus rendering ibrutinib a reversible inhibitor of BTK. (58-60) Despite the decreased BTK binding affinity, ibrutinib still allows for partial disease control in the presence of *BTK* p.Cys481Ser via competitive binding; however, imminent disease

progression may necessitate an alternative treatment strategy to prevent or overcome a potential clinical relapse in patients harbouring this genetic alteration. (61)

Cysteine-to-serine mutation in BTK at the ibrutinib-binding cysteine residue was first identified by Woyach and colleagues in five patients with progressive CLL treated with ibrutinib. The authors performed whole exome sequencing of samples obtained at the time of clinical relapse. (58) Since then, multiple studies from clinical trials and international research groups as well as our research group have confirmed *BTK* p.Cys481Ser as the predominant alteration driving ibrutinib resistance and implied the mutation as a potential biomarker candidate for molecular disease monitoring in ibrutinib-resistant CLL. (54, 56, 62) However, temporal dynamics of the *BTK* p.Cys481Ser and correlations between the emergence of the resistance mutation and disease progression are still largely unknown.

I.3.5. The role of the Bcl-2 inhibitor venetoclax in CLL

B cell lymphoma 2 (Bcl-2) protein family plays a pivotal role and are key regulators of apoptosis. (63) Disturbing the delicate balance of pro-apoptotic and pro-survival Bcl-2 proteins and shifting toward the latter is an endorsed mechanism of tumorigenesis by cancer cells evading programmed cell death. Profound genetic changes, notably translocations, affecting *BCL2*, the founding member of the Bcl-2 family, have been previously described in follicular lymphoma, however its therapeutic significance became particularly apparent in CLL with first-in-class Bcl-2 inhibitor venetoclax restoring the apoptotic function and changing the treatment paradigm of the disease.

Venetoclax is a BH3 mimetic agent delivering remarkable results with CR rates up to 30-50% through direct inhibition of the pro-apoptotic Bcl-2. (64-67) Currently, venetoclax-based treatment options include continuous monotherapy or fixed duration venetoclax *plus* anti-CD20 obinutuzumab combination for one year in front-line cases or venetoclax *plus* rituximab combination for two years in R/R cases. (65, 66, 68) Unlike with ibrutinib, based on initial results from two clinical trials, a dose escalation scheme was established to prevent life-threatening tumour lysis syndrome with a weekly dose ramp-up to 400 mg per day of venetoclax in both continuous and time-limited treatment setups. (64, 69)

In a pivotal phase 1/2 clinical trial by Roberts and colleagues, continuous single agent venetoclax therapy induced and sustained CR in 20% of a heavily pre-treated patient

population with 89% of the patients having clinical or genetic risk factors associated with poor prognosis. (64) These results were later confirmed by another clinical trial conducted by Stilgenbauer and colleagues in 107 patients with R/R CLL harbouring del(17p). (70) In this multicentre phase 2 study, ORR was 79.4% after a median 12.1 months of follow up, suggesting continuous venetoclax monotherapy as an encouraging and potent therapeutic option for patient with very poor prognostic features.

The combination of venetoclax *plus* rituximab was first investigated in 49 patients with R/R CLL by Seymour and colleagues, with ORR and CR of 86% and 51%, respectively. (71) In the phase 3 MURANO trial comparing venetoclax *plus* rituximab to rituximab *plus* bendamustine combination including 389 cases with R/R disease, patients received venetoclax for up to two years complemented by rituximab in the first 6 months of therapy. (65, 72) At the 5-year follow up, median PFS was 53.5 months in the venetoclax *plus* rituximab cohort with a 5-year OS of 82.1%. (73) These results together established the fixed duration venetoclax *plus* rituximab combination as a new promising second-line treatment in CLL.

Venetoclax *plus* obinutuzumab combination was initially evaluated within the framework of the pivotal phase 3 CLL14 study involving 12 previously untreated patients with coexisting conditions and showed an impressive ORR of 100% and undetectable ($<10^{-4}$) minimal residual disease (MRD) in the peripheral blood in all cases. (66) Selected patients received venetoclax for up to one year completed by obinutuzumab in the first 6 month of therapy. Full cohort analysis and follow up of 432 patients from the CLL14 protocol showed a 4-year PFS of 74% in the venetoclax-obinutuzumab arm with median PFS not reached. (74) Importantly, response to venetoclax *plus* obinutuzumab combination therapy seemed independent from poor prognostic features, such as *TP53* disruption and unmutated IGHV. An additional important finding of the CLL14 trial was the very high rate of undetectable MRD remissions achieved in 76% of the venetoclax-treated patients. (66)

Data from sizeable cohort studies suggest venetoclax as a potent novel targeted agent with favourable toxicity pattern with most common adverse effect being mild or manageable neutropenia and infections. (10) Overall, both continuous and time-limited inhibition of Bcl-2 by venetoclax is an encouraging and today widely used treatment

strategy in CLL, achieving remarkable results, namely $<10^{-4}$ MRD negativity, in older and unfit patients as well as in patients with poor prognostic features in both front-line and R/R settings.

I.3.6.Molecular background of resistance to venetoclax

Despite the notable response rates across numerous studies, durable remission is eventually followed by loss of efficacy and subsequent progressive CLL in a subset of patients. (64, 65) Acquired or secondary venetoclax resistance occurs in both continuous and time-limited treatment setups and is the leading cause of treatment failure. Over the past few years, a spectrum of genetic, epigenetic and transcriptional changes underlying venetoclax resistance has been observed *in vitro* as well as under clinical circumstances. (75)

Molecular mechanisms reported to be associated with secondary resistance include changes in the pro-survival gene *BCL2* diminishing venetoclax binding. So far, a handful of mutations have been demonstrated to confer resistance by reducing or inhibiting binding affinity of venetoclax to Bcl-2. Glycine-to-valine substitution emerging at position 101 in the gene *BCL2* mitigates the response to venetoclax *in vitro* via the modulation of the BH3 binding domain and is associated with clinical resistance in up to 50% of the cases with progressive CLL. *BCL2* p.Gly101Val was first reported by Blombery and colleagues in 7 venetoclax-resistant patients. (76) Several additional *BCL2* mutations, such p.Asp103Tyr or p.Asp103Gln directly disrupting the BH3 binding P4 pocket, have been observed in CLL patients establishing a complex co-operating mutational pattern with co-existing *BCL2* mutations driving venetoclax resistance. (77-80) Interestingly, Kater and colleagues unveiled recurrent mutations in genes *BIRC3*, *BRAF*, *TP53*, *NOTCH1* and *XPO1* but no *BCL2* within the framework of the MURANO trial. Mutations emerging in *BCL2* associated with acquired resistance have been observed only in continuous treatment, suggesting a putative alternative mechanism driving leukemogenesis and progression in time-limited settings. (73)

The molecular changes underlying acquired venetoclax resistance further encompass overexpression of the pro-survival BCL-XL, Bcl-2, MCL1 and XIAP, and the emergence of complex karyotype. (75, 81-89) Mitochondrial metabolic reprogramming has also been

described as a putative driver mechanism of venetoclax resistance through AMPK-regulated oxidative phosphorylation in venetoclax-refractory CLL. (75, 87, 90)

Although the proportion of CLL clones harbouring genetic, epigenetic and translational changes associated with venetoclax resistance varies widely from smaller subclones to large tumour compartments, subclonal presence and cumulative abundance of genetically and/or translationally altered leukemic cells are reported in the majority of the cases. (79) Intriguingly, subclonality of mutation-bearing or anti-apoptotic protein-overexpressing CLL cells have been observed even in patient samples obtained at the time of clinical relapse implying the significant role of co-operating genetic and epigenetic events, and CLL cell plasticity in evading venetoclax-induced apoptosis resulting in overt disease progression. (76, 80-82) Recently, this multi-layered adaptation enabling CLL relapse during venetoclax therapy has been further dissected by Thijssen and colleagues unveiling ubiquitous NF-kB activation in CLL cells and suggesting MCL1 as a direct transcriptional target of NF-kB. (82)

I.4. Overcoming resistance hurdles in CLL

Significance of the early detection of resistance to BTK and Bcl-2 inhibition has been increasing with the growing number of alternative therapeutic options available for patients relapsing on targeted therapies. Monitoring of distinct genetic, epigenetic and transcriptional changes conferring resistance to targeted agents allows for adequate disease control using alternative classes of targeted drugs.

Patients with ibrutinib failure can be treated with agents targeting oncogenic pathways PI3K-mTOR and Bcl-2. Ongoing trials and real-world datasets evaluating the efficacy of subsequent Bcl-2 inhibition by venetoclax in ibrutinib-resistant CLL cases have reported remarkable outcomes with the Bcl-2 antagonist conferring massive reduction of CLL cells resistant to BTK inhibition. (91, 92) Further treatment strategies to tackle ibrutinib resistance include the application of reversible BTK inhibitors effective against CLL cells regardless of their *BTK* Cys481 status, SYK inhibitors, BTK degraders as well as CAR-T cells. (93-96) Acquired venetoclax resistance may potentially be overcome by subsequent covalent BTK inhibition using ibrutinib. So far, a handful of cohort studies and the follow up from the MURANO study in ibrutinib-naïve CLL patients

discontinuing venetoclax have concluded that the efficacy of post-venetoclax BTK inhibition did not seem to be altered by prior Bcl-2 antagonist treatment. (84, 97-100)

Although sequential resistance to both ibrutinib and venetoclax conferring dismal outcomes and poor prognosis may occur in some cases, venetoclax *plus* ibrutinib combination therapy and venetoclax re-treatment are interesting approaches to address double class-resistant cases. (101-103) Combination therapy is also a promising chemotherapy-free proposal in both R/R and treatment-naïve patients without a prior history of resistance. Considering the updated data from the phase 2 CLARITY trial investigating time-limited venetoclax *plus* continuous ibrutinib in R/R settings, the combination can provide synergistic activity targeting different CLL subpopulations, with CR rates comparable to other venetoclax-based regimens. (104, 105)

DOI:10.14753/SE.2023.2857

II. OBJECTIVES

With a series of studies, we aimed to unveil distinct genetic changes underlying resistance to targeted therapies in CLL and to scrutinize the correlation between the emergence of selected gene mutations and relapse or disease progression. We collected and analysed specimens from CLL patients treated with ibrutinib or venetoclax with special emphasis on pre-progression and progression samples to assess the clinical value and feasibility of the sensitive screening for the most frequent resistance mutations. Our data may have implications for the sensitive monitoring of targeted therapies which may allow for the early detection of an impending relapse and the identification of patients who could potentially benefit from change of therapy.

Specific objectives:

- To develop a droplet digital polymerase chain reaction (ddPCR)-based method for the detection of the most frequent resistance mutations conferring secondary resistance to ibrutinib or venetoclax therapy
- To dissect the temporal heterogeneity observed in R/R CLL in the context of ibrutinib therapy by the molecular monitoring of the *BTK* p.Cys481Ser mutation
- To unveil clonal evolution during venetoclax therapy by the molecular screening for the most frequent variants *BCL2* p.Gly101Val and p.Asp103Tyr, as well as other pathogenic *BCL2* mutations by targeted next-generation sequencing (NGS)
- To assess the correlation between acquired ibrutinib resistance and the emergence of *BTK* p.Cys481Ser
- To assess the correlation between acquired venetoclax resistance and selected *BCL2* mutations
- To aid the standard-of-care monitoring of the efficacy of ibrutinib and venetoclax therapies in CLL

III. MATERIALS

III.1. Patient samples

III.1.1. Samples obtained from patients treated with ibrutinib

Serial PB samples were collected from 83 R/R CLL patients (49 males and 34 females; median age at diagnosis: 57 years, range: 26-85 years) treated with ibrutinib. (60) This cohort comprised a subset of 126 patients investigated within the framework of the Hungarian Ibrutinib Resistance Analysis Initiative (MIRA). Selected patients received single agent ibrutinib in a daily dose of 420 mg for a minimum of 13 months (median 36 months, range: 13-68 months). Our cohort represented a heavily pre-treated patient population with a median 2 lines (range: 1-6 lines) of prior therapy. Median follow up time was 40 months (range: 13-69 months) with over 90% of the samples collected prospectively. Progressive disease and relapse were defined as the emergence of at least one novel symptom based on the iwCLL criteria for active disease (4) and change of therapy was issued at the physician's discretion. Considering patients with progressive CLL on ibrutinib, samples obtained prior to the first clinical signs of disease progression were available in 56.8% (25/44) of the cases, while the first follow-up specimen of 19 patients was received at the time of CLL relapse or progression. Follow-up specimens from patients carrying *BTK* p.Cys481Ser and/or progressing on ibrutinib were collected with a median interval of 4 months (range: 1-11 months) allowing for temporal monitoring of the resistance mutation. Clinical characteristics are summarised in Supplementary Table 1.

III.1.2. Samples obtained from patients treated with venetoclax

Peripheral blood samples were collected from 67 patients (46 males and 21 females) with R/R CLL treated with venetoclax monotherapy or venetoclax in combination with rituximab. (106) Fixed duration venetoclax *plus* rituximab combination was applied in 23.9% (16/67) of the patients, with time-limited and continuous venetoclax monotherapy administered in 7.5% (5/67) and 68.6% (46/67) of the cases, respectively. At the time of last follow up, 11 patients have stopped the time limited venetoclax treatment after a median 26 months (range: 7-32 months) due to subsequent deep remission. Similar to our cases treated with ibrutinib, this CLL patient cohort also comprised a subset of patients with prior BTK inhibition who had been investigated within the framework of the nation-

wide MIRA project. Following the ramp-up period, selected patients received venetoclax in a daily dose of 400 mg for a median 20 months (1-83 months) complemented with rituximab during the first 6 months of therapy in cases with fixed duration treatment setup. Our cohort represented a pre-treated patient population with a median of 2 lines (range: 1-6 lines) of prior therapy. Median follow-up time was 23 months (range: 1-83 months) with samples obtained at the time of last follow up in patients with no clinical signs of disease progression. Progressive disease and relapse were defined as previously described. In cases with progressive CLL, samples were collected at the time of clinical relapse or progression after a median 14 months (range: 1-48 months) of venetoclax therapy. Follow-up samples obtained from patients harbouring co-existing *BCL2* mutations revealed by ultra-deep targeted sequencing were collected as part of the routine diagnostic work-up and were analysed retrospectively. Clinical characteristics are summarised in Supplementary Table 2.

III.1.3. Molecular, cytogenetic and flow cytometry analyses

Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation from the PB specimens following the assessment of leukemic cell purity by flow cytometry using CD5/CD19/CD45 immunophenotypic markers. In cases with low whole blood cell counts (i.e. low white blood cell count), leukemic cell purity was assessed using CD19/CD5/CD22/CD43/CD79b/CD81/CD3/ROR1 immunophenotypic markers following the most recent ERIC guideline on minimal residual disease monitoring in CLL. (107) All reported mutation allelic burden values were normalised to the leukemic cell fraction assessed by flow cytometry. DNA isolation was performed using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Basel, Switzerland) following the manufacturer's instructions. After DNA extraction, *TP53* mutations and the IGHV mutation status were analysed according to the most recent ERIC guidelines. (19, 108) Chromosomal abnormalities, including del(11q), del(13q) and del(17p), as well as the trisomy of chromosome 12, were investigated by FISH using dual-colour Vysis probe sets (Abbott Molecular, Illinois, USA). PBMCs from five and three healthy volunteers were used as negative controls in *BTK* and *BCL2* ddPCR mutation analyses, respectively. Written informed consent was obtained from every participant. Both studies were approved by the Hungarian Medical Research Council (ID: 5495-3/2021/EKU) and were conducted in accordance with the Declaration of Helsinki.

III.2. Droplet digital PCR

Screening and quantitative assessment of the *BTK* p.Cys481Ser (single tube mutant/wild-type allele assay: dHsaMDS802598840) and selected *BCL2* resistance mutations (p.Gly101Val, no Bio-Rad ID; p.Asp103Tyr, no Bio-Rad ID; p.Asp103Glu, single tube mutant/wild-type allele assay: dMDS433558996; p.Ala113Gly, mutant allele assay: dHsaMDW3410683143, wild-type allele assay: dHsaMDM3410683141; p.Val156Asp, single tube mutant/wild-type allele assay: dHsaMDS355891150) were screened by ddPCR using assays designed for the discriminative analysis of mutant and wild-type alleles. (50, 106) Reactions were carried out on the QX200 Droplet Digital PCR platform (Bio-Rad Laboratories, California, USA) according to the manufacturer's instructions with the recommended 90-100 ng of input DNA. Allelic burden of each mutation was defined as fractional abundance (FA) calculated from the ratio of the number of mutant DNA molecules (a) and the total number of mutant (a) plus wild-type (b) DNA molecules detected: $FA = \frac{a}{a+b}$. FA values were normalised to the CLL cell fraction measured by flow cytometry. Quantitative reliability of the assays was determined with linearity measurements using dilution series and the sensitivity of the ddPCR analysis was assessed for each sample from the final droplet counts. The median lower limit of the quantitative range could ubiquitously be determined as 0.01% FA.

III.3. Ultra-sensitive next-generation sequencing

Targeted ultra-deep NGS was performed on follow-up samples of ibrutinib-treated patients harbouring the wild-type *BTK* Cys481 allele and progressing on ibrutinib, as well as samples of venetoclax-treated patients with progressive CLL obtained at the time of relapse or disease progression. NGS was carried out using a QIAseq Targeted DNA Custom Panel (Qiagen, Hilden, Germany) covering four genes (*BTK*, *PLCG2*, *BCL2* and *TP53*) relevant to ibrutinib or venetoclax therapy and resistance. Libraries were prepared according to the manufacturer's recommendations and sequenced on a MiSeq platform (Illumina, California, USA) with 150 bp paired-end configuration. Data processing and bioinformatic analyses were performed with the smCounter2 workflow utilising unique molecular identifier-based variant calling facilitating a highly accurate detection of low-frequency variants. (109) Variants were annotated using the dbSNP, COSMIC, ClinVar,

SnpSift and SnpEff databases with additional gene-specific The TP53 Database (originally IARC database) and Seshat databases used for the annotation of somatic *TP53* mutations. (110, 111) Reported variant allele frequencies (VAF) were normalised to the leukemic cell fraction assessed by flow cytometry.

III.4. Statistics

GraphPad Prism 9.1.0 (GraphPad Software, California, USA) was utilised for calculating median values and confidence intervals (CI), as well as for analysing the cumulative incidence of disease progression in patients harbouring the *BTK* p.Cys481Ser resistance mutation, and for performing the Mantel–Cox test to compare PFS and OS between patients harbouring *BCL2* resistance and cases with wild-type *BCL2* Gly101 and Asp103. The Mantel–Byar estimate of PFS in patients with or without detectable *BTK* p.Cys481Ser resistance mutation was calculated by R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

IV. RESULTS

IV.1. Molecular screening and monitoring of ibrutinib resistance

IV.1.1. Molecular and cytogenetic characteristics of the ibrutinib study cohort

Molecular and cytogenetic features considered as independent prognostic markers in CLL [del(11q), del(13q), del(17p), trisomy 12, *TP53* mutations, IGHV mutation status] were screened as part of the standard-of-care diagnostic work-up (Supplementary Table 1). *TP53* mutation status screened by ultra-deep NGS and del(17p) status analysed by FISH were available in 75/83 and 81/83 of the ibrutinib-treated cases, respectively. Further cytogenetic abnormalities identified by FISH, such as del(11q), del(13q) and trisomy 12, were identified with a frequency of 15.7%, 26.5% and 12.0%, respectively. IGHV mutation status was available in 79 cases with 87.3% of them carrying IGHV-U. High risk genetic features conferring adverse prognosis were identified in 89.2% (74/83) patients, which together with the failure of prior therapy lines is a strong indicator that our selected patients adequately represented a real-world R/R patient cohort typically selected for ibrutinib therapy.

IV.1.2. Ultra-sensitive screening for the BTK p.Cys481Ser resistance mutation

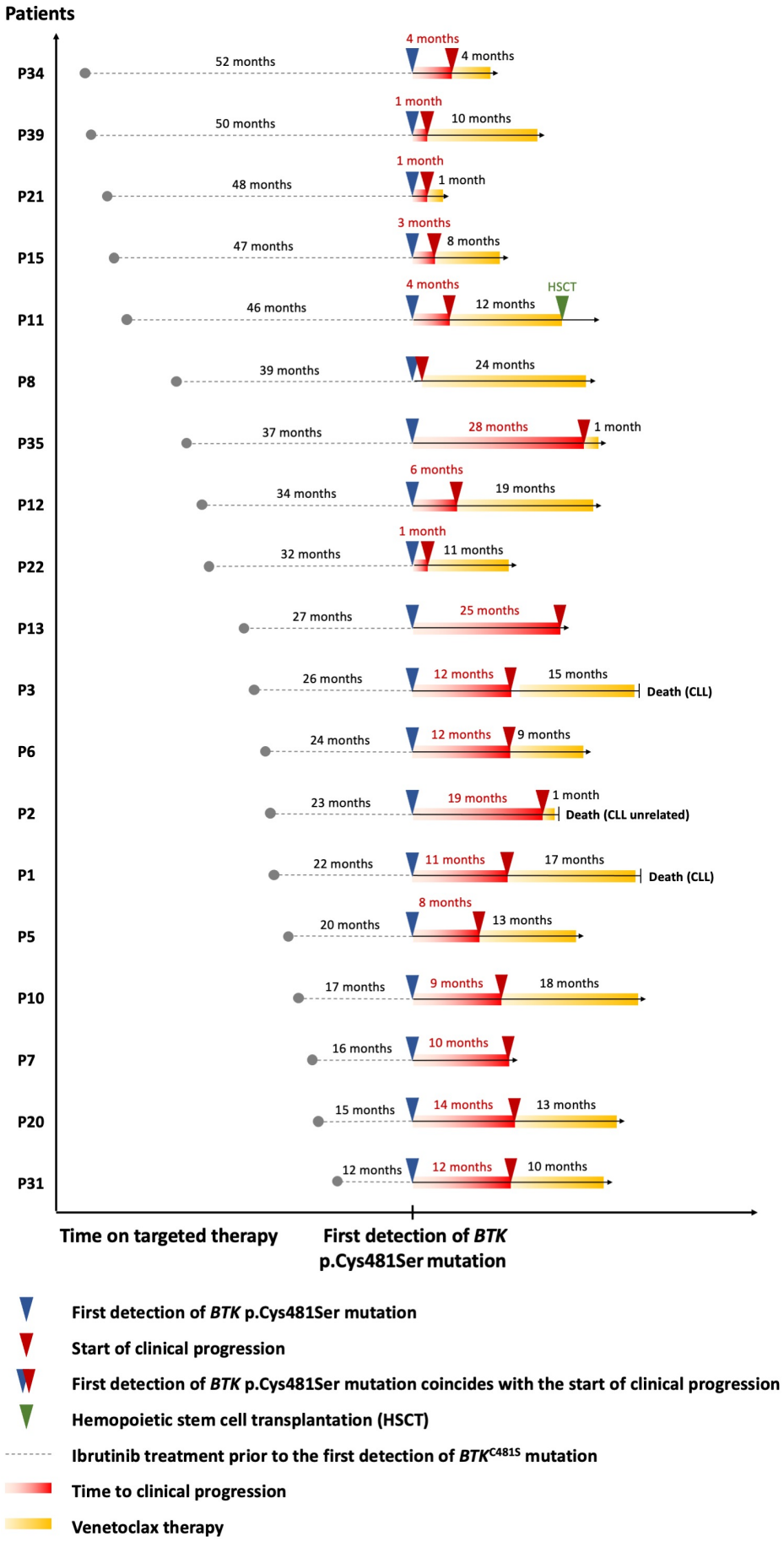
Screening for the *BTK* p.Cys481Ser mutation associated with acquired ibrutinib resistance was performed both retro- and prospectively on a total of 305 PB-derived samples using a highly sensitive (10^{-4}), custom-designed locus specific ddPCR assay. With a median follow up of 40 months (range: 13-69 months), *BTK* p.Cys481Ser was detected in 48.2% (40/83) of the patients with 80.0% (32/40) of them experiencing relapse or disease progression during the follow up period. These 32 patients represented 72.7% (32/44) of all cases with progressive CLL or relapse. Median FA value at the time of relapse or disease progression was 10.66% (range: 0.01-90.00% ; 95% CI: 3.0-23.0%). Richter's transformation was not observed among the 40 patients harbouring *BTK* p.Cys481Ser.

IV.1.3. Time-resolved monitoring of the BTK p.Cys481Ser mutation

The 32 patients carrying detectable *BTK* p.Cys481Ser and experiencing acquired ibrutinib resistance underwent disease progression after median 38 months (range: 13-65 months; 95% CI: 32.0-44.0 months) of ibrutinib therapy. In 19 patients with samples obtained

prior to disease progression, emergence of the resistance mutation preceded the first clinical signs of ibrutinib failure with median 9 months (range: 0-28 months; 95% CI: 3.0-12.0 months) as shown in Figure 4 and 5. Cumulative incidence of disease progression in patients with pre-progression samples available for monitoring and harbouring *BTK* p.Cys481Ser is depicted in Figure 6A.

DOI:10.14753/SE.2023.2857



DOI:10.14753/SE.2023.2857

▲ **Figure 4.** Treatment timeline of 19 patients with relapsed or therapy refractory chronic lymphocytic leukemia carrying the *BTK* p.Cys481Ser ibrutinib resistance mutation and experiencing relapse or disease progression during the follow up period. Blue wedges denote the timepoint of the emergence of *BTK* p.Cys481Ser with red wedges indicating the first clinical observation of relapse or disease progression. First detection of the resistance mutation predated the first clinical signs of progression with median 9 months (range: 0-28 months) as indicated by the red bars. Subsequent venetoclax therapy was applied in 89.5% (17/19) of the patients represented by yellow bars.

Median FA values of *BTK* p.Cys481Ser at the time of first detection versus at the time of relapse or disease progression were 0.385% versus 10.66%, demonstrating a median 28-fold increase in the allelic burden of the resistance mutation (Figure 6B).

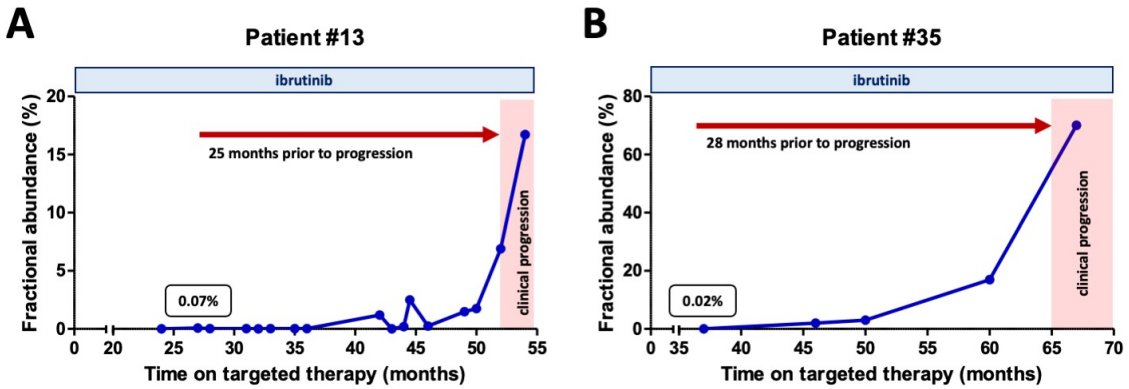


Figure 5. Temporal dynamics of the *BTK* p.Cys481Ser resistance mutation in two patients with relapsed or therapy refractory chronic lymphocytic leukemia treated with ibrutinib. (A) In Patient #13, the resistance mutation was first detected after 27 months of ibrutinib therapy with a fractional abundance value (FA) of 0.07%. Emergence of *BTK* p.Cys481Ser preceded the first clinical observation of disease progression by 25 months. In the last sample obtained at the time of progression *BTK* p.Cys481Ser was detected with an FA value of 16.7% demonstrating a 239-fold increase in the allelic burden of the mutation. (B) In Patient #35, emergence of the *BTK* p.Cys481Ser mutation was observed after 37 months of BTK inhibition with an FA value of 0.02% and its first detection predated the first clinical signs of disease progression by 28 months. The mutation with gradually increasing allelic burden was detectable across the samples received during the examined period. In the latest sample obtained two months after the first clinical observation of progression, *BTK* p.Cys481Ser was detected with an FA value of 70%, representing a 3,500-fold increase in the allelic burden of the mutation compared to its first detection.

DOI:10.14753/SE.2023.2857

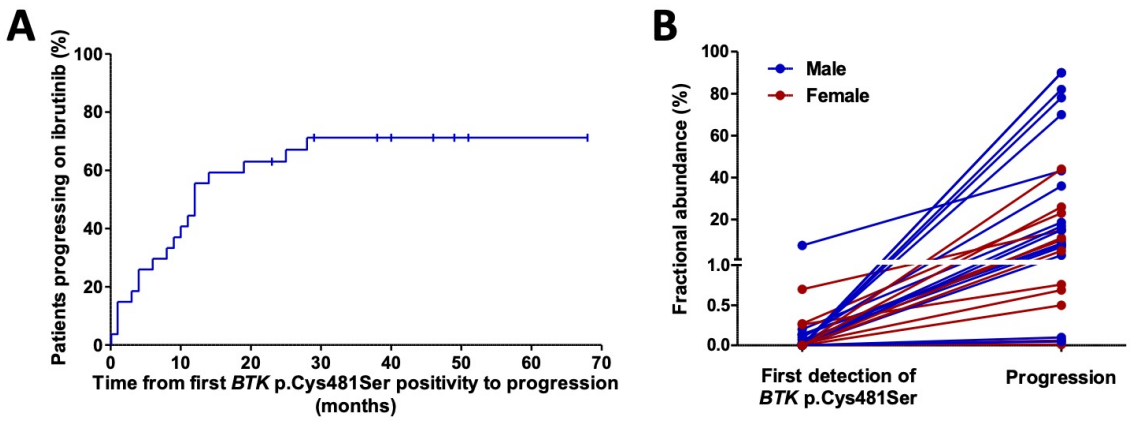


Figure 6. Disease progression and fractional abundance (FA) of the *BTK* p.Cys481Ser mutation in patients harbouring the variant. (A) Cumulative incidence of chronic lymphocytic leukemia (CLL) progression after the emergence of *BTK* p.Cys481Ser. (B) FA values of *BTK* p.Cys481Ser in individual patients at the time of first detection *versus* at the time of relapse or disease progression. FA values of the resistance mutation showed an increasing tendency ubiquitously across the cases, including 11 male and 8 female patients. Patients with an allelic burden of the X chromosome-linked *BTK* gene mutation higher than 50% at the time of disease progression were all males.

Mantel–Byar test was performed to analyse CLL progression on ibrutinib with all patients starting in the *BTK* Cys481 wild-type group and being transferred to the *BTK* p.Cys481Ser mutant group upon first detection of the resistance mutation. A significantly inferior PFS rate was observed among patients acquiring *BTK* p.Cys481Ser compared to cases with wild-type *BTK* Cys481 (p value <0.00001; Figure 7).

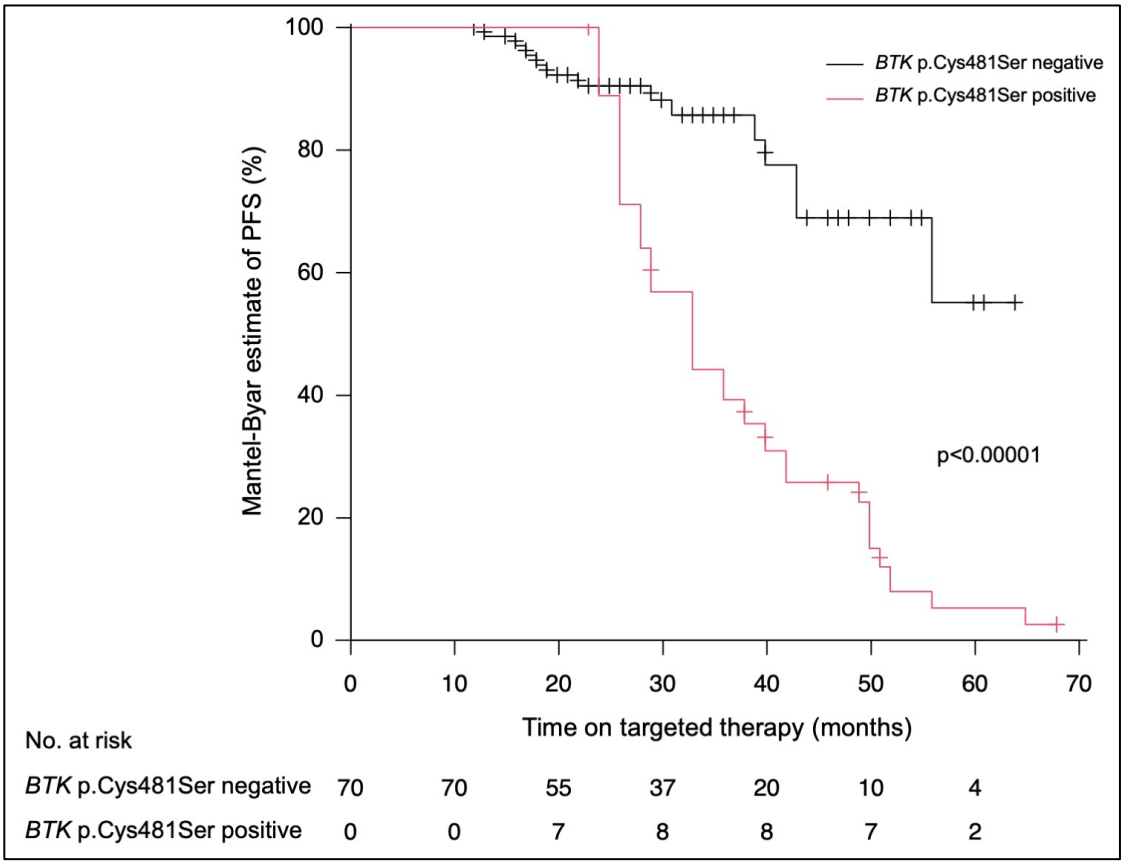


Figure 7. Disease Mantel–Byar estimate of progression-free (PFS) survival from the first detection of BTK p.Cys481Ser. Patients experiencing disease progression with samples obtained prior to relapse and cases with no clinical signs of progression were included in the analysis.

IV.1.4.Management of secondary ibrutinib resistance in patients with BTK p.Cys481Ser

Due to ibrutinib failure and subsequent disease progression, 87.5% (28/32) of the patients carrying *BTK* p.Cys481Ser have discontinued ibrutinib treatment. Successive Bcl-2 inhibition by venetoclax was applied in all these cases resulting in clinical remission and elimination of the *BTK* p.Cys481Ser mutant CLL subclones within median 3 months (range: 2-7 months) across the patients. Despite the remarkable and durable response rates and decrease in the allelic burden of *BTK* p.Cys481Ser (Figure 8A), 8 patients experienced relapse or disease progression, and developed secondary venetoclax resistance during the follow-up period (Figure 8B). Six out of these 8 patients have succumbed to their disease without receiving any subsequent therapy, while two patients are still alive to date with one of them undergoing hematopoietic stem cell transplantation and the other patient receiving salvage chemoimmunotherapy.

DOI:10.14753/SE.2023.2857

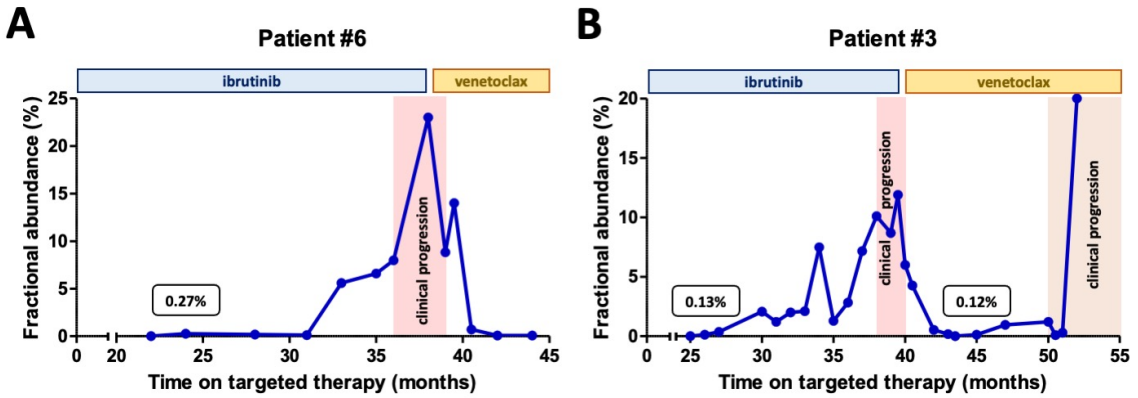


Figure 8. Temporal dynamics of the *BTK* p.Cys481Ser mutation in two patients with relapsed or therapy refractory chronic lymphocytic leukemia treated with ibrutinib and subsequent Bcl-2 inhibitor venetoclax therapies. (A) In the longitudinally collected samples of Patient #6 *BTK* p.Cys481Ser was first detected after 24 months of ibrutinib therapy with an FA value of 0.27%, predating the first clinical observation of disease progression with 12 months. In the sample obtained during the ramp-up period of subsequent venetoclax therapy was observed followed by a sharp decrease in the allelic burden of the mutation. Mutation-bearing CLL cells were eliminated by the Bcl-2 inhibitor therapy and the *BTK* p.Cys481Ser resistance mutation was undetectable in the follow-up samples obtained during the 6 months of venetoclax therapy. The patient was still alive and well at the time of last follow-up and showed no signs of relapse or disease progression. (B) *BTK* p.Cys481Ser in Patient #3 was detected after 26 months of ibrutinib treatment preceding the first clinical signs of disease progression by 12 months. After ibrutinib failure, subsequent venetoclax therapy was administered resulting in a sharp decrease the FA values of the resistance mutation. Despite the abrupt reduction of the allelic burden of *BTK* p.Cys481Ser, recurrence of the resistance mutation was observed after 5 months of venetoclax therapy predating a second disease progression by 5 months. The patient eventually developed acquired venetoclax resistance with an increasing FA value of *BTK* p.Cys481Ser and succumbed to her disease 5 months after the first clinical signs of venetoclax failure.

IV.1.5. Patients harbouring the BTK p.Cys481Ser mutation with no signs of disease progression

BTK p.Cys481Ser resistance mutation was detected in 8 patients with no clinical evidence of relapse or disease progression during the follow up period (Figure 9). These cases represent 20.0% (8/40) and have continued ibrutinib therapy with the mutation regularly monitored by ddPCR. At the latest follow up timepoint, the median FA value of the resistance mutation was 0.69% (range: 0.20%) after median 43 months (range: 23-68 months) of ibrutinib therapy. Intriguingly, emergence of the *BTK* p.Cys481Ser was later followed by the complete elimination of the acquired mutation in 3 cases. Quantitative ddPCR analyses of the sequential samples from the remaining 5 patients unveiled an

increasing tendency of the FA values of the *BTK* p.Cys481Ser resistance mutation, highlighting the significance of the clinical and molecular monitoring of these patients for signs of an impending relapse. Two patients carrying *BTK* p.Cys481Ser and not experiencing relapse or disease progression died from reasons unrelated to CLL during the examined period, with last measured *BTK* p.Cys481Ser FA values of 0.08% and 19.1%, respectively (Figure 9).

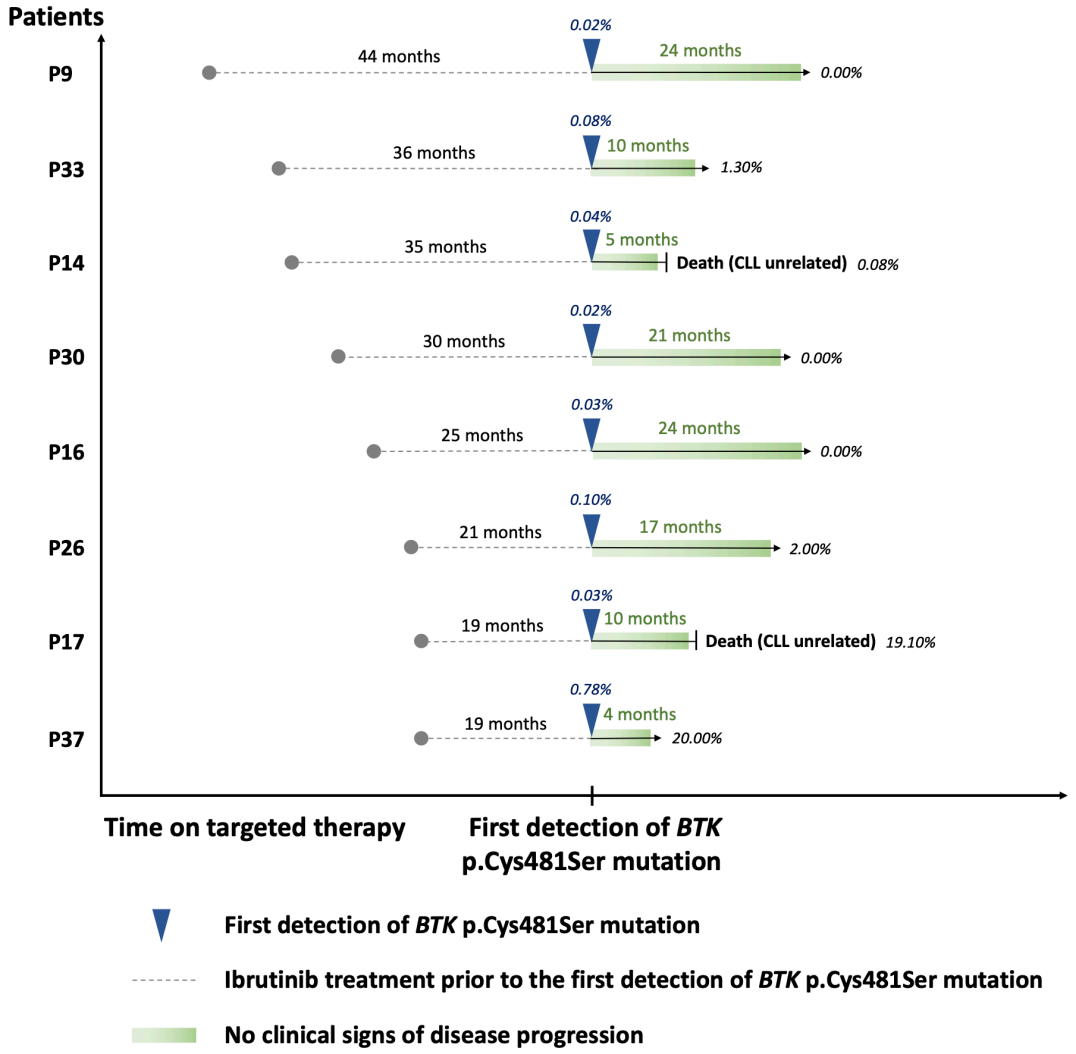


Figure 9. Treatment timeline of 8 patients harbouring *BTK* p.Cys481Ser with no clinical evidence of disease progression during the study period. In this cohort, representing 20% (8/40) of the patients, emergence of the mutation was detected after median 28 months (range: 19-44 months) of ibrutinib therapy as denoted by blue wedges. Fractional values (FA) of *BTK* p.Cys481Ser in blue represent the allelic burden of the mutation at the time of first detection, while black FA values denote the allelic burden at the time of last follow-up.

IV.1.6.Secondary ibrutinib resistance in patients with wild-type BTK Cys481

Twelve patients progressing on ibrutinib harboured wild-type *BTK* Cys481 assessed by ddPCR. This patient group, representing 27.3% of the patients experiencing relapse (12/44), exhibited the first clinical signs of relapse or disease progression after median 26 months (range: 13-56 months) of ibrutinib therapy. As an attempt to investigate and unveil alternative genomic aberrations leading to acquired ibrutinib resistance, targeted ultra-deep sequencing covering all coding regions of the *BTK*, *PLCG2* and *TP53* genes with a median depth of 4,133× (range: 3,390-13,856×) was performed on samples obtained from these patients within median 1.5 months (range: 0-7 months) of the first clinical signs of disease progression. Ultra-deep NGS uncovered a novel *PLCG2* gene mutation in the TIM (X-box) domain of the protein in one patient with no additional mutation affecting the *BTK* and/or *PLCG2* genes in the analysed patients. The observed *PLCG2* p.Asp334Gly (c.1001A>G) variant is a previously unreported finding in ibrutinib-resistant R/R CLL; however, the aspartic acid-to-glycine substitution at the Asp334 residue has already been found to be associated with Richter's transformation by Maddocks and colleagues. (52) Somatic variants detected by ultra-deep NGS in patients having wild-type *BTK* Cys481 and progressing on ibrutinib are listed in Supplementary Table 3.

IV.2. Molecular monitoring of venetoclax resistance

IV.2.1.Molecular and cytogenetic characteristics of the venetoclax study cohort

Similar to our cases treated with ibrutinib, independent cytogenetic and molecular prognostic biomarkers were assessed as part of the routine diagnostic workflow (Table 6). Del(17p) status and *TP53* mutation positivity were available in 94.0% (63/67) and 83.6% (56/67) of the cases, respectively with patients harbouring del(17p) and/or *TP53* mutation(s) comprising 40.3% (27/67) of the analysed patient population. Additional cytogenetic aberrations including del(11q), del(13q) and trisomy of chromosome 12 were identified in 22.4% (15/67), 22.4% (15/67) and 11.9% (8/67) of the cases, respectively. IGHV mutation status was assessed in 52 patients, with 82.7% (43/52) of them carrying unmutated IGHV (IGHV-U). High-risk genetic features associated with adverse prognosis, including *TP53* aberrations and IGHV-U status, were observed in 83.6%

(56/67) of the study cohort, which together with the failure of prior therapy lines depicted a real-world R/R patient population typically selected for venetoclax therapy.

IV.2.2.Sensitive screening for BCL2 p.Gly101Val and p.Asp103Tyr

Screening for *BCL2* p.Gly101Val and p.Asp103Tyr was performed on samples obtained from 67 R/R CLL patients using locus-specific ddPCR assays designed and optimized for the sensitive (10^{-4}) interrogation of these two mutational hotspots. *BCL2* p.Gly101Val and p.Asp103Tyr were detected in 10.4% (7/67) and 11.9% (8/67) of the cases, respectively, with 4 patients harbouring both mutations. The two resistance mutations were first observed after median 15 months (range: 5-48 months) of venetoclax therapy. Ten out of eleven (90.9%) patients carrying p.Gly101Val and/or p.Asp103Tyr experienced relapse or disease progression, representing 43.5% (10/23) of all cases developing secondary venetoclax resistance during the follow-up period. Richter's transformation was observed in one patient tested positive for *BCL2* p.Gly101Val and in another patient with wild-type Gly101 and Asp103 alleles after 15 and 25 months of venetoclax therapy, respectively. Intriguingly, all *BCL2* p.Gly101Val and p.Asp103Tyr variants were detected in patients receiving venetoclax as a continuous single-agent treatment, while the two resistance mutations were not observed during or after fixed duration venetoclax therapy. Median FA values of p.Gly101Val and p.Asp103Tyr at the time of sample collection were 0.29% (range: 0.047%-7.8%) and 0.28% (range: 0.018%-8.94%), respectively. Median FA value of *BCL2* p.Asp103Tyr at the time of sample collection in four cases with wild-type G101 was 0.023% (range: 0.015-0.16%). Since *BCL2* p.Gly101Val and p.Asp103Tyr were exclusively detected in patients with continuous venetoclax therapy, cases with time-limited treatment setup were excluded from the PFS and OS analyses. In the analysed patient population, significantly inferior PFS as defined by the iwCLL 2018 criteria (4) was observed among patients carrying *BCL2* p.Gly101Val and/or p.Asp103Tyr mutations as compared to cases with wild-type *BCL2* Gly101 and Asp103 loci (Mantel–Cox test, p value = 0.0192) (Figure 10A). No significant difference in OS rates was observed between the two patient populations with subsequent lines of therapy applied in both cohorts (Mantel–Cox test, p value = 0.6904) (Figure 10B). *TP53* mutation positivity and number of prior therapy lines did not influence the PFS in the analysed patient cohort (Figure 10C and D).

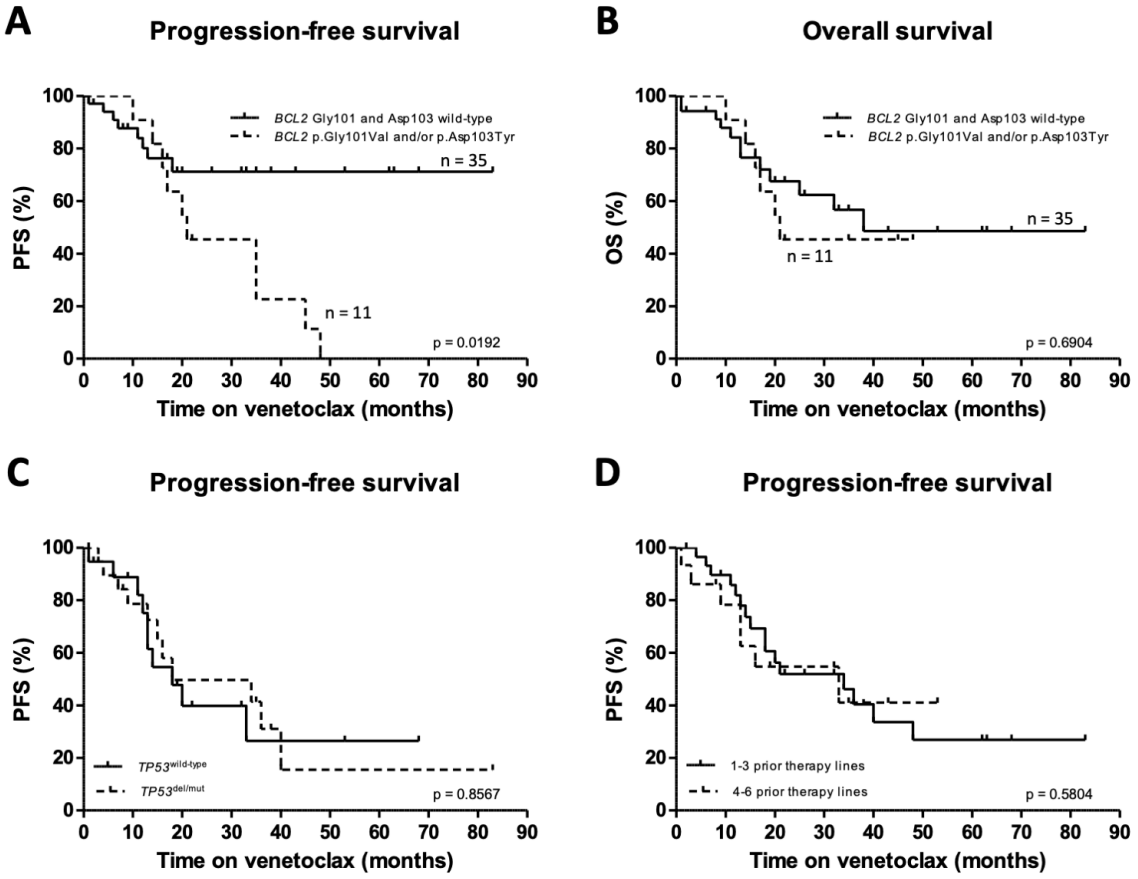


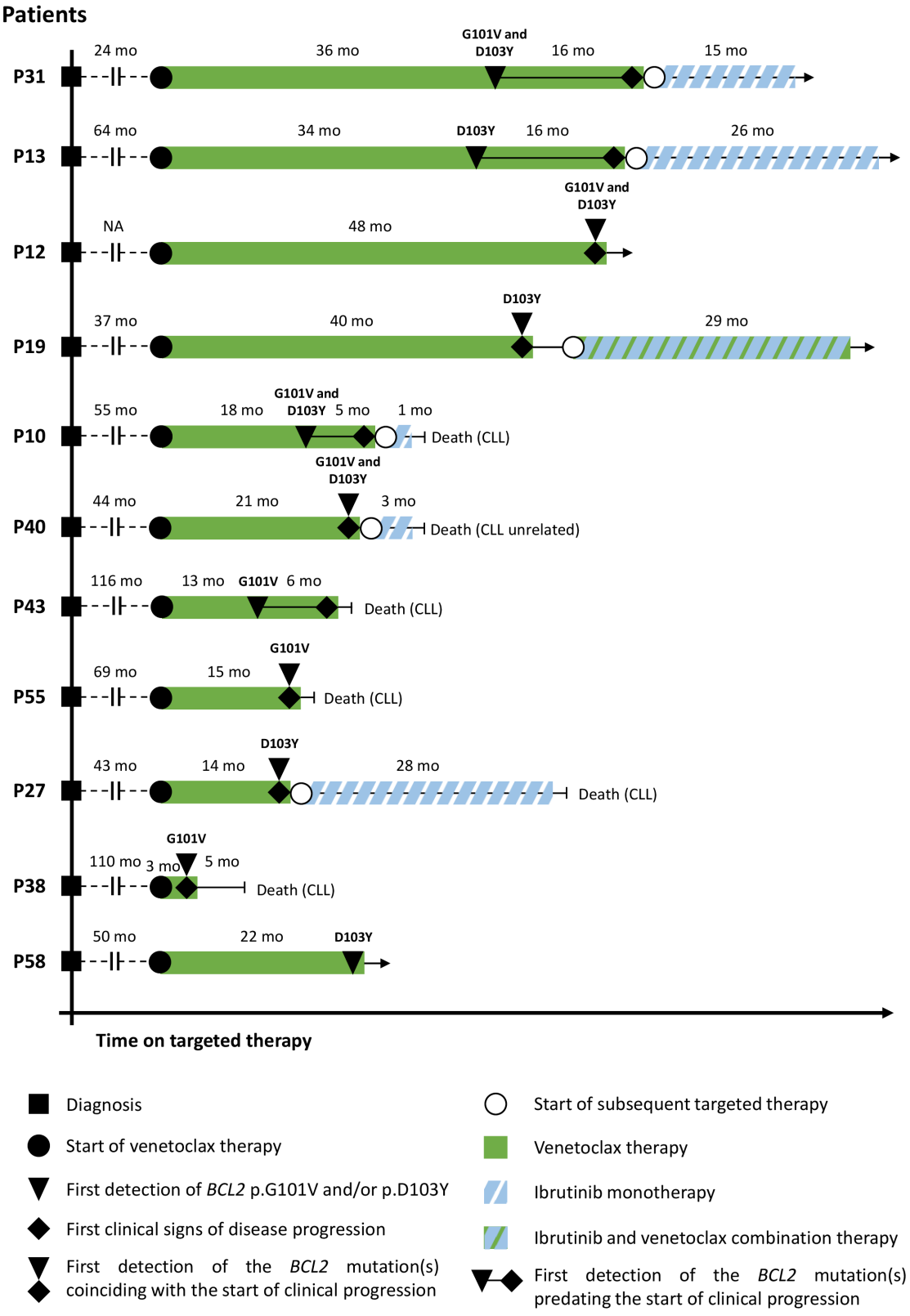
Figure 10. Progression-free survivals (PFS) and overall survival (OS) of patients with relapsed or therapy refractory chronic lymphocytic leukemia (CLL) receiving continuous venetoclax therapy. (A) PFS was significantly shorter in patients harbouring *BCL2* p.Gly101Val and/or p.Asp103Tyr compared to *BCL2* Gly101 and Asp103 wild-type cases. Data were censored at the time of last follow-up in patient not experiencing disease progression. (B) While a trend towards shorter was also observed among patients with *BCL2* p.Gly101Val and/or p.Asp103Tyr, no statistical significance has been reached between the two populations. (C) No significant difference was observed in the PFS of patients carrying *TP53* disruptions (deletion of 17p and *TP53* mutations) compared to cases with wild-type *TP53.* (D) The number of prior therapy lines (1-3 *versus* 4-6) did not significantly influence the PFS in CLL patients with continuous venetoclax therapy.

IV.2.3.Management of secondary venetoclax resistance in patients with p.Gly101Val and/or p.Asp103Tyr

Due to acquired venetoclax resistance and subsequent disease progression, 90.9% (10/11) of the patients harbouring BCL2 p.Gly101Val and/or p.Asp103Tyr have discontinued single agent venetoclax after median 21 months (range: 10-48 months) of Bcl-2 inhibition (Figure 11). Successive BTK inhibition by ibrutinib was administered in 60% (6/10) of

DOI:10.14753/SE.2023.2857

the cases, from which one patient received ibrutinib in combination with continued venetoclax treatment.



▲ **Figure 11.** Treatment timeline of 11 patients treated with continuous venetoclax therapy and harbouring *BCL2* p.Gly101Val and/or p.Asp103Tyr. Ten out of 11 patients experienced relapse or disease progression during the follow-up period with six of them receiving subsequent ibrutinib monotherapy or salvage ibrutinib plus venetoclax combination. In 4 cases with samples obtained prior to disease progression, emergence of the *BCL2* p.Gly101Val and/or p.Asp103Tyr predated the first clinical signs of relapse with a median of 11 months (range: 6-16 months). One patient (P58) carrying *BCL2* p.Asp103Tyr was still alive and well at the time of last follow-up without showing any clinical signs of disease progression after 22 months of venetoclax therapy.

Despite the temporary regression in clinical symptoms across the patients, three patients receiving salvage ibrutinib developed secondary ibrutinib resistance after median 28 months (range: 1-29 months) of BTK inhibition, with two out of six patients succumbing to their disease without receiving any further treatment. One patient developing detectable *BCL2* p.Gly101Val and p.Asp103Tyr mutations on venetoclax and receiving subsequent ibrutinib therapy died from reasons unrelated to CLL during the follow-up period. Three out of 10 mutation-bearing patients progressing on venetoclax have died without receiving any subsequent therapy, with one out of 3 patients undergoing Richter's transformation. One patient harbouring *BCL2* p.Gly101Val was still alive to date at the time of last follow-up after 22 months of continuous venetoclax monotherapy with no clinical signs of relapse or disease progression.

IV.2.4.Additional BCL2 mutations revealed by targeted next-generation sequencing

Ultra-deep targeted sequencing was performed on 18 samples obtained at the time of relapse or disease progression, irrespective of their *BCL2* Gly101 and Asp103 mutation status previously assessed by ddPCR. The investigated patient population comprised of 9 cases with *BCL2* p.Gly101Val and/or p.Asp103Tyr, and a subset (9 patients) of patients with wild-type Gly101 and Asp103 developing secondary venetoclax resistance after median 15 months (range: 1-48 months) of targeted therapy. Targeted NGS was performed covering all coding regions of *BTK, PLCG2, BCL2* and *TP53* with a median depth of 4,242× (range: 942-18,476×). NGS uncovered three additional *BCL2* mutations, namely p.Asp103Glu, p.Ala113Gly, p.Val156Asp in four cases. Notably, all four patients were previously found to harbour *BCL2* p.Gly101Val and/or p.Asp103Tyr as assessed by ddPCR, with no *BCL2* variants unveiled in *BCL2* Gly101 and Asp103 wild-type cases. Somatic variants uncovered by targeted NGS in samples collected at the time of disease progression are listed in Supplementary Table 4.

IV.2.5. Time-resolved monitoring of co-occurring BCL2 resistance mutations

Four patients harbouring multiple *BCL2* mutations relapsed or experienced disease progression after median 29 months (range: 18-48 months) of continuous single agent venetoclax therapy (Figure 11). Quantitative ddPCR using locus-specific and custom-designed assays was performed on longitudinally collected samples of all four cases allowing for the retrospective temporal dissection of the additionally detected *BCL2* variants p.Asp103Glu, p.Ala113Gly and p.Val156Asp. In samples obtained at the time of relapse or disease progression, median FA values of *BCL2* p.Gly101Val, p.Asp103Tyr, p.Asp103Glu, p.Ala113Gly and p.Val156Asp were 0.50% (range: 0.16-2.20%), 1.15% (range: 0.05-3.40%), 1.16% (range: 0.37-1.95%), 0.37% (range: 0.05-5.76%), and 2.20%, respectively. In all cases with samples obtained prior to progression, emergence of *BCL2* p.Gly101Val and p.Asp.103Tyr, as well as co-occurring *BCL2* mutations preceded the first clinical signs of disease progression with median 11 months (range: 5-16 months) (Figure 12A-C, Patient #43 harbouring exclusively p.Gly101Val mutation is not shown). Allelic burdens of *BCL2* p.Gly101Val, p.Asp103Tyr, p.Asp103Glu, p.Ala113Gly and p.Val156Asp were increased at the time of relapse as compared to samples collected prior to disease progression, demonstrating a median of 6.61-fold (range: 1.2-18.75) increase in the FA values of each mutation over time (Figure 13).

IV.2.6. Secondary venetoclax resistance in cases with wild-type BCL2

Thirteen patients progressing on venetoclax harboured wild-type *BCL2* Gly101 and Asp103 as assessed by ddPCR. This subset of patients represented 56.5% of the cases developing secondary venetoclax resistance after median 13 months (range: 1-33 months) of Bcl2 inhibition. Interestingly, all patients experiencing relapse or disease progression received venetoclax as a continuous single agent therapy. Subsequent salvage chemo-immunotherapy was applied in 2 cases. In one patient, successive rituximab was applied in combination with continued venetoclax, and in one case, continuous venetoclax monotherapy was complemented by salvage ibrutinib therapy. Eight patients have succumbed to their disease after developing secondary venetoclax resistance without receiving any subsequent lines of treatment. As an attempt to unveil alternative molecular mechanisms underlying acquired venetoclax resistance, ultra-deep targeted sequencing was performed on samples obtained at the time of disease progression available in 9 cases

with wild-type *BCL2*Gly101 and Asp103 as described previously. Interestingly, no further resistance mutations affecting the *BCL2* gene were identified in any of the analysed cases suggesting the presence of alternative genomic lesions localized outside the coding region of *BCL2*, or the emergence of epigenomic/transcriptomic changes eventually leading to manifest venetoclax resistance.

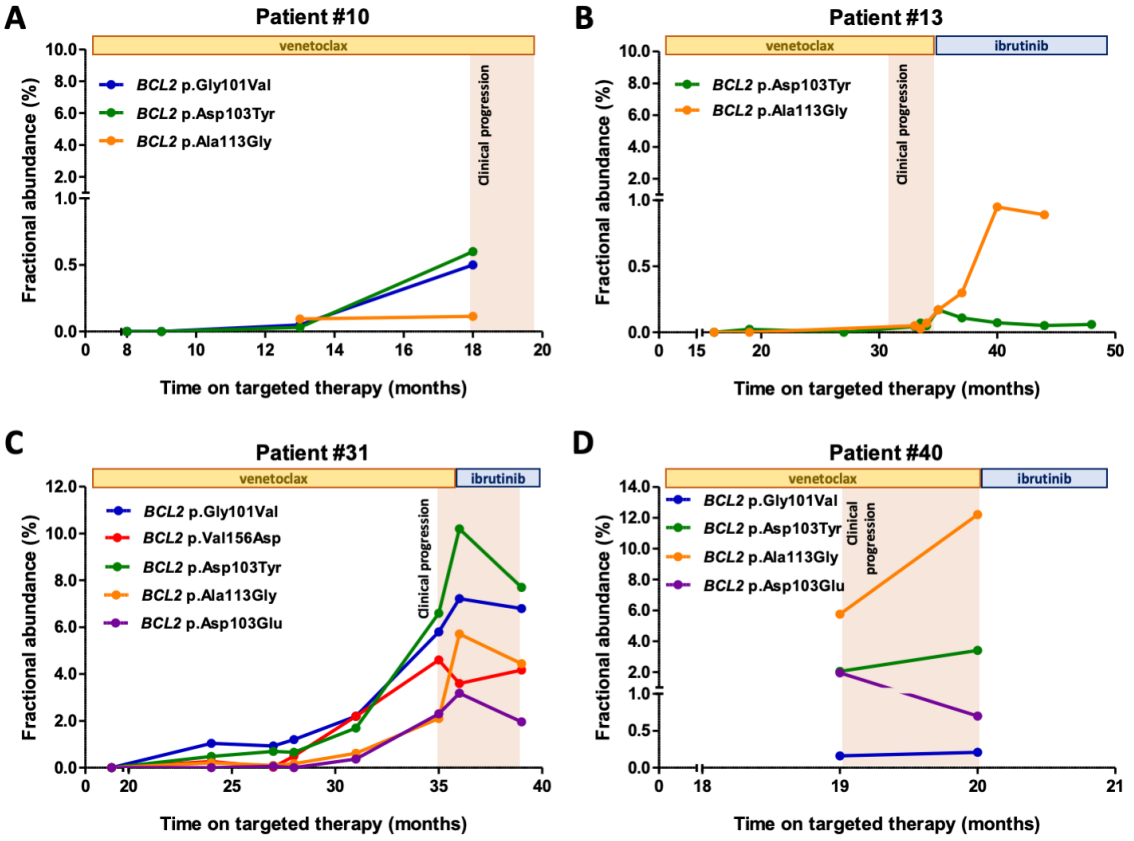


Figure 12. Temporal dynamics of *BCL2* mutations in 4 patients harbouring multiple variants associated with venetoclax resistance. (A) In Patient #10, *BCL2* p.Gly101Val and p.Asp103Tyr were first observed after 13 months of continuous single-agent venetoclax therapy with fractional abundance (FA) values of 0.05% and 0.03%, respectively. Additionally, a co-existing subclonal variant p.Ala113Gly was identified by ultra-sensitive droplet digital PCR (ddPCR). First detection of the 3 mutations predated the first clinical signs of disease progression with 5 months. (B) Patient #13 harboured *BCL2* p.Asp103Tyr detected by ddPCR with an FA value of 0.17%. The mutation was successfully backtracked in samples collected prior to disease progression. Targeted sequencing uncovered a co-existing p.Ala113Gly variant in the sample obtained after venetoclax failure. (C) In a sample obtained from Patient #31 at the timepoint of disease progression following 35 months of venetoclax therapy, both *BCL2* p.Gly101Val and p.Asp103Tyr were observed with FA values of 5.8% and 6.6%, respectively. Targeted sequencing of the same sample unveiled three additional co-existing variants, namely p.Ala113Gly, p.Asp103Glu and p.Val156Asp with CCF values of 2.1%, 2.3% and 6.6%, respectively. All variants excluding p.Asp103Glu were successfully backtracked in longitudinally collected samples obtained 16 months prior to disease progression.

Interestingly, *BCL2* p.Asp103Glu was first observed after 27 months of Bcl-2 inhibition suggesting multi-layered subclonal changes driving secondary venetoclax resistance. Subsequent ibrutinib was administered following the onset of clinical progression resulting in an observable decline in the allelic burden of all *BCL2* variants. (D) Patient #42 harboured four distinct *BCL2* variants, namely p.Gly101Val, p.Asp103Tyr, p.Ala113Gly and p.Asp103Glu observed in the sample collected at the time of disease progression with CCF values of 0.21%, 3.4%, 12.2% and 0.7%, respectively.

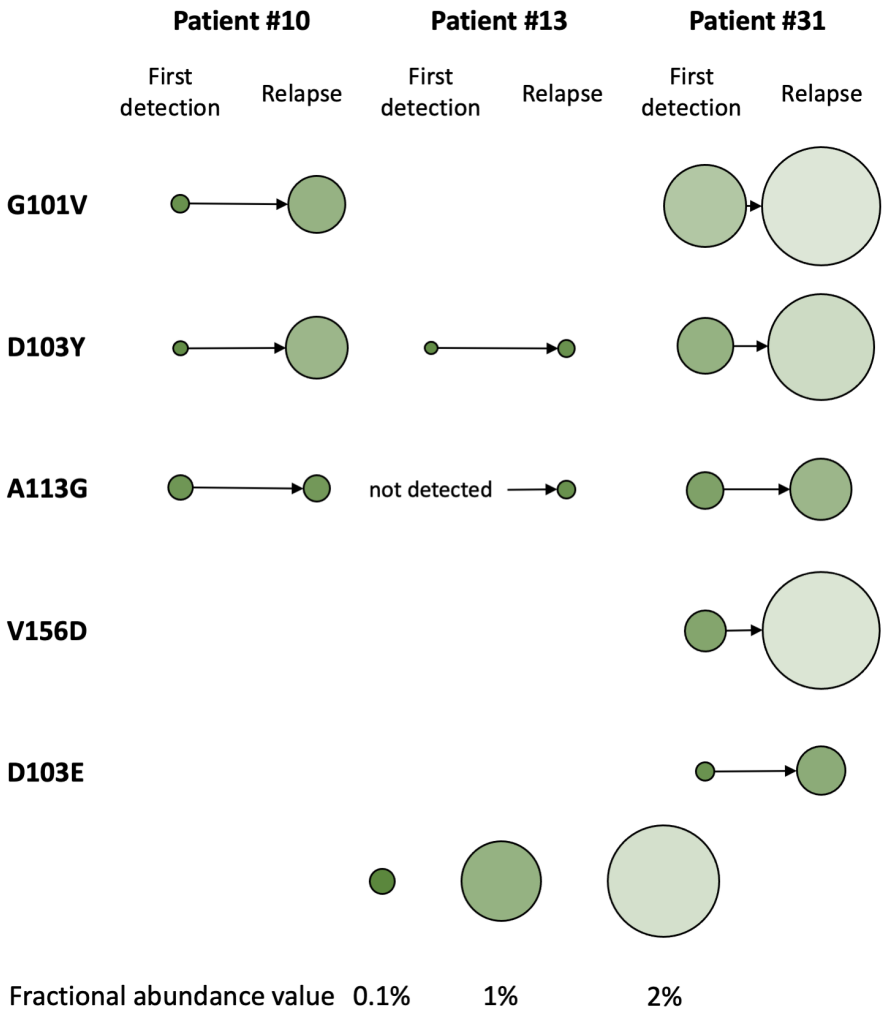


Figure 13. Fractional abundance (FA) values of *BCL2* variants in patients with samples obtained prior to disease progression *versus* at the time of relapse. Allelic burden of p.Gly101Val, p.Asp103Tyr, p.Ala113Gly, p.Val156Asp and p.Asp103Glu exhibited an increased tendency ubiquitously across the 3 analysed patients. Diameter and colour of the circles are proportional to the FA values of each mutation in individual samples. In Patient #13, *BCL2* p.Ala113Gly mutation was first detected in the samples obtained at the timepoint of disease progression.

V. DISCUSSION

Targeted therapies, particularly the Btk inhibitor ibrutinib and the Bcl-2 inhibitor venetoclax, have revolutionized the therapeutic landscape of R/R CLL with high response rates and prolonged PFS in heavily pre-treated patient populations. (35-37, 64, 65, 71) Despite the remarkable improvement in long-term clinical outcomes, loss of efficacy is still a major clinical concern as subsequent relapse or progressive CLL eventually occurs in a subset of patients treated with targeted agents. (53, 58, 84, 92, 112) Indeed, since the failure of ibrutinib and venetoclax confers dismal prognosis without additional or subsequent salvage therapy, there is a high clinical interest in predicting relapse or disease progression, which could potentially be addressed by molecular screening for recurrent resistance mutations in longitudinally collected patient samples.

V.1. Sensitive screening for resistance mutations in ibrutinib- and venetoclax-treated patients

In the framework of a nationwide research initiative, we investigated the feasibility and clinical value of screening for the most common resistance mutations underlying acquired ibrutinib and venetoclax resistance in R/R CLL patient populations using an ultra-sensitive ddPCR-based method. In 83 ibrutinib-treated R/R CLL patients, representing the largest real-world cohort reported at the date of publishing in which *BTK* p.Cys481Ser has been monitored longitudinally, the mutation was detected in nearly half of the cases with 80% of the mutation-bearing patients showing signs of disease progression. Although we only interrogated this single aberration, our approach uncovered the underlying resistance mutation and provided informative genetic data for molecular monitoring in nearly 73% of the patients undergoing relapse or disease progression. (60) Similar to our results, Woyach and colleagues investigated 46 R/R patients progressing on ibrutinib from four clinical trials and identified *BTK* and/or *PLCG2* mutations in 87% of the relapse samples. (53) Ahn and colleagues analysed CLL patients carrying *TP53* disruptions in a phase 2 clinical trial and detected *BTK* and/or *PLCG2* mutations in 80% (8/10) of the patients with progressive CLL. (54) In a real-world cohort of CLL patients receiving ibrutinib for a minimum of 3 years, Quinquenel and colleagues performed a

'snapshot' screening of *BTK* p.Cys481Ser and found that the presence of the variant was strongly associated with progressive disease. (56)

In a real-world cohort of 67 patients with R/R CLL treated with single agent venetoclax therapy or venetoclax *plus* rituximab combination treatment, we performed 'snapshot' screening of the two most frequent *BCL2* mutations associated with resistance. Using an ultra-sensitive ddPCR method, *BCL2* p.Gly101Val and/or p.Asp103Tyr were identified in 16.4% of the analysed patient population with some 90% of affected patients showing signs of relapse or disease progression during the follow-up period. (106) This subset of mutation-carriers represented over 40% of the patients experiencing progressive CLL. Notably, in our cohort, all patients harbouring detectable *BCL2* mutations could have been identified with the exclusive screening for *BCL2* p.Gly101Val and p.Asp103Tyr, demonstrating the potential clinical value of ddPCR testing targeting these variants during venetoclax therapy.

In summary, with the ultra-sensitive ddPCR-based analyses of one mutation hotspot associated with ibrutinib resistance and two hotspots linked to venetoclax failure, we successfully identified the underlying molecular mechanisms in two-thirds (32/44 and 10/23 of all ibrutinib- and venetoclax-resistant cases, respectively) of the patients undergoing relapse or disease progression during targeted treatments. Our results imply the selected gene mutations as promising potential molecular biomarkers for use in the standard-of-care diagnostic work-up of targeted therapy resistance in CLL.

V.2. Co-operating subclonal mutational patterns in venetoclax-treated R/R CLL

To unveil alternative or co-operating genetic changes driving acquired venetoclax resistance, targeted ultra-deep NGS was performed on specimens obtained at the time of disease progression, revealing three additional *BCL2* mutations p.Asp103Glu, p.Ala113Gly and p.Val156Asp in four cases. These mutations were previously found to be co-occurring with *BCL2* p.Gly101Val and were associated with resistance to Bcl-2 inhibition *in vivo*. (78, 113) Interestingly, no additional *BCL2* variants were uncovered in cases with wild-type *BCL2* Gly101 and Asp103 as assessed by ddPCR. All patients harbouring multiple *BCL2* mutations displayed co-operating subclonal mutational patterns with total allelic burdens of less than 10%, in all but one case even in samples

obtained at the time of relapse. Plausible explanations for this phenomenon may include the key role of mutually exclusive mutation-bearing subclones reported by Thompson and colleagues, as well as the ability of mutation-bearing cells to promote resistance in *BCL2* unmutated CLL subclones. (83)

Our findings are in line with previous studies performed on patient cohorts from various clinical trials. Anderson and colleagues investigated 67 patients with R/R CLL receiving venetoclax in three early-phase trials and identified *BCL2* p.Gly101Val in 11 cases with all patients ultimately experiencing relapse or disease progression. (79, 84) Consistent with our observations, allelic burden of the mutation ranged from 0.1% to 68.7% displaying significant heterogeneity within the analysed patient cohort. In the same patient population, Blombery and colleagues uncovered median 3 additional recurrent mutations affecting *BCL2* including p.Asp103Glu, p.Ala113Gly and p.Val156Asp in all but one case using digital and hybrid capture-based NGS approaches. (79, 83) Similarly, Lucas and colleagues have reported recurrent *BCL2* mutations p.Ala113Gly, p.Phe104Leu, p.Phe104Ser, p.Leu119Val, p.Gly101Ala and the internal tandem duplication p.Arg107_Arg110 co-existing with p.Gly101Val in a cohort of 24 patients with ibrutinib- and venetoclax-resistance. (80) Together with our observations of additional acquired *BCL2* variants, these studies provide a growing body of evidence of the branching subclonal composition of venetoclax-resistant tumor compartments in individual patients, and highlight the frequent presence of multiple, independent mechanisms driving clinical resistance. (82, 83)

Among the *BCL2* variants unravelled by ddPCR or targeted sequencing, substitutions emerging at Asp103 are particularly noteworthy. The Asp103 residue is located in the P4 pocket of Bcl-2 establishing a hydrogen bond with venetoclax. (69) Missense mutations affecting the Asp103 impart the affinity of Bcl-2 for venetoclax without disrupting the protein's pro-survival function and its affinity for BH3-only proteins as observed by Blombery and colleagues. (79) In the same study, substitutions p.Asp103Glu, p.Asp103Val and p.Asp103Tyr were detected across 6 patients with R/R CLL. *BCL2* p.Asp103Tyr was first described by Tausch and colleagues as a co-operating mutation in driving acquired venetoclax resistance in a single patient who was previously found to harbour p.Gly101Val. (77) In our patient population, p.Asp103Tyr was identified in 8 patients, of which 4 patients carried p.Asp103Tyr in the absence of p.Gly101Val. To our

knowledge, here we first reported the recurrent *BCL2* p.Asp103Tyr as a sole alteration affecting *BCL2* underpinning venetoclax failure, further underlining the role of the Asp103 residue in secondary venetoclax resistance.

Intriguingly, no *BCL2* variants were observed among patients receiving time-limited venetoclax therapy either as a single agent treatment or in combination with rituximab. This finding is in line with the results of a handful of previous studies, including the 4-year data from the phase 3 MURANO trial by Kater and colleagues. (65, 72) The authors analysed end of treatment samples of 194 patients treated with fixed duration venetoclax *plus* rituximab combination therapy and identified recurrent mutations in genes *BIRC3*, *BRAF*, *TP53*, *NOTCH1* and *XPO1* with no variants affecting *BCL2*.

V.3. Temporal dissection of mutations underlying acquired ibrutinib and venetoclax resistance

In patients progressing on ibrutinib with samples obtained prior to progression, emergence of the resistance mutation *BTK* p.Cys481Ser predated the first clinical signs of ibrutinib failure with median 9 months and in 79% (15/19) of the cases, the mutation was detectable at least 3 months prior to relapse or disease progression. Consequently, the presence and increasing abundance of *BTK* p.Cys481Ser proved to be a good indicator of an impending relapse well before the first clinical signs of relapse or disease progression in the majority (79% × 73%) of our patients with informative follow up. Similarly, Woyach and colleagues have reported that the emergence of the detectable mutation preceded the time of relapse with an estimated 9.3 months in 20 patients with serial samples analysed retrospectively. (53) In the patient cohort investigated by Ahn and colleagues, 6/8 patients had detectable *BTK* and/or *PLCG2* mutations predating the clinical progression by 8 months. (54) Based on the results of a preliminary study by our research group, *BTK* and *PLCG2* mutations were backtracked in five patients treated with ibrutinib and were detectable on average 10.5 months prior to progression. (50) These studies together provide a growing body of evidence that *BTK* p.Cys481Ser is not only the predominant driver alteration conferring acquired ibrutinib resistance, but its temporal dynamics over the disease course may also imply the mutation as an important biomarker candidate for molecular disease monitoring in ibrutinib-treated CLL patients.

Retrospective analysis and temporal dissection of the uncovered *BCL2* variants in longitudinally collected samples of patients receiving venetoclax revealed heterogeneous and dynamically changing subclonal FA values of mutations during venetoclax therapy. This suggests a complex underlying architecture of resistant CLL cell compartments, coupled with subsequent progressive CLL in all four cases analysed longitudinally. To our knowledge, these patients represent the first longitudinally monitored and temporally dissected cases with venetoclax resistance to date.

V.4. Management of secondary ibrutinib and venetoclax resistance

Significance of detecting targeted therapy resistance at an early stage has been exponentially increasing with the growing number of alternative treatment options entering the clinical practice. As previously described, patients with ibrutinib failure can be successfully treated with alternative classes of targeted agents, such as PI3K antagonists, BTK degraders, CAR-T cell therapy and venetoclax with the latter option being routinely used in every-day patient care. Bcl-2 inhibition by venetoclax in patients progressing on ibrutinib has conferred massive reduction of CLL cells resistant to BTK inhibition in our cohort, which is in line with data reported by other research groups in both clinical trials and real-world settings. (91, 92) Subsequent treatments of choice in cases with acquired venetoclax resistance include irreversible BTK inhibition by ibrutinib, applied in 60% of the cases harbouring *BCL2* mutations and experiencing relapse or disease progression in our cohort, with half of them delivering durable responses. Consistent with our observations, a number of studies with small number of patients and a cohort study by Mato and colleagues concluded that the efficacy of BTK inhibition therapy does not seem to be altered by prior venetoclax treatment. (97-99)

Although in our patient populations only one patient with venetoclax failure received ibrutinib *plus* venetoclax combination therapy achieving durable disease control, this combination along with venetoclax re-treatment is a promising approach to address cases with resistance to either of these targeted agents. Considering the data from the phase 2 CLARITY trial, chemotherapy-free, time-limited venetoclax *plus* ibrutinib therapy demonstrated remarkable clinical outcomes in R/R CLL patients providing synergistic activity by targeting distinct CLL subpopulations. (104, 105)

V.5. Biological and technical challenges in the molecular monitoring of therapy resistance

The role of molecular testing for mutations during ibrutinib and venetoclax treatment is still an intensively studied research area with our limited understanding posing a handful of compelling technical and biological challenges. (A) Not all mutation-bearing patients relapse or experience disease progression, though the proportion of cases with stable disease will most likely decrease with longer follow-up times available in the future. (B) CLL cell populations may be heterogeneous harbouring distinct genetic, epigenetic and transcriptional changes and the interaction between these subclones and their contribution to clinical progression are still poorly understood. This challenge is particularly peculiar in venetoclax-treated patients with complex subclonal architecture of multiple *BCL2* mutations in distinct resistant CLL compartments. (C) Most of the patients progressing on venetoclax and some cases with ibrutinib failure harbour resistance mutations in minor subclones and the abundance of mutations in the PB specimens does not always correlate with relapse or disease progression. (79, 114) A plausible explanation for this phenomenon has been described in the context of venetoclax treatment by Thompson and colleagues implying the role of mutually exclusive *BCL2* mutant subclones, however further aetiologies in both BTK and Bcl-2 inhibition, such as the role of permissive microenvironment, mutant CLL cells promoting resistance in non-mutated malignant cells and compartment effect, have also been proposed by us as well as other research groups. (53, 62, 83) (D) Finally, around one-third of the patients with progressive disease analysed in our cohorts carried wild-type *BTK* Cys481 and *BCL2*. Several alternative genetic and non-genetic changes mechanisms have been described as potential contributors to both ibrutinib or venetoclax resistance, however further clinical and functional studies are required to clarify their causative role in targeted agent failure.

In summary, our series of studies demonstrate the feasibility and clinical value of robustly detecting the most frequent *BTK* p.Cys481Ser mutation underlying acquired ibrutinib resistance and the two most common *BCL2* mutations p.Gly101Val and p.Asp103Tyr associated with secondary resistance under real-world circumstances. To our knowledge, our patient cohorts were the largest real-world R/R CLL patient populations at the time

of publishing, in which resistance mutations have been molecularly monitored during Btk or Bcl-2 inhibition. Based on the presented data, screening for these alterations can greatly facilitate the early detection of resistance in up to two-thirds of the patients receiving targeted therapies and experiencing relapse or disease progression. Although longer follow up times and prospective studies on independent patient cohorts are needed to refine the predictive power of these ddPCR tests, the clinical value of such analyses in the state-of-the-art patient care will probably increase in the future. Additional genomic and non-genomic studies, preferably coupled with functional readouts, will further contribute to our deepened understanding of key mechanisms orchestrating the interplay between CLL compartments driving resistance to targeted BTK and Bcl-2 inhibition. Ultimately, our gradually expanding knowledge of resistance mechanisms will hopefully further improve the clinical management of patients with R/R CLL treated with targeted agents and experiencing relapse or disease progression.

DOI:10.14753/SE.2023.2857

VI. CONCLUSIONS

Novel findings of my thesis are:

- Sensitive ddPCR-based methods are optimal for the early detection of an impending relapse as well as for the identification of underlying molecular mechanisms in up to two-thirds of R/R CLL patients treated with targeted agents experiencing relapse or disease progression.

- In R/R CLL patients progressing on ibrutinib with PB specimens obtained prior to ibrutinib failure, emergence of the underlying resistance mutation *BTK* p.Cys481Ser predated the first clinical observation of acquired ibrutinib resistance with median 9 months. In the majority of the cases using sensitive ddPCR, the mutation was detectable at least 3 months prior to relapse or disease progression.

- We unveiled genetic alterations conferring resistance to the Bcl-2 inhibitor venetoclax by the molecular screening for the most frequent underlying variants *BCL2* p.Gly101Val and p.Asp103Tyr in nearly half of the patients with venetoclax failure and subsequent disease progression.

- We dissected the temporal heterogeneity observed in R/R CLL in the context of venetoclax therapy by the molecular monitoring of pathogenic *BCL2* variants using ddPCR and ultra-deep targeted NGS. Convergent evolution of distinct mutation-bearing CLL subclones was observed in the longitudinally collected samples of four patients.

DOI:10.14753/SE.2023.2857

VII. SUMMARY

Targeted therapies, such as the Btk inhibitor ibrutinib and the Bcl-2 inhibitor venetoclax, have revolutionized the therapeutic landscape of relapsed or therapy refractory (R/R) chronic lymphocytic leukemia (CLL). However, despite the notable response rates across patient populations with adverse cytogenetic, molecular or clinical features, durable remission is eventually followed by loss of efficacy and subsequent progressive CLL in a subset of patients conferring poor prognosis.

In our study we performed droplet digital polymerase chain reaction (ddPCR)-based methods for the sensitive detection of the most common resistance mutations underlying ibrutinib and venetoclax failure in 83 and 67 R/R CLL patients treated with ibrutinib and venetoclax, respectively. In the ibrutinib-treated cohort, acquired resistance-associated mutation *BTK* p.Cys481Ser was detected in 40 patients with 80.0% (32/40) of them experiencing relapse or disease progression during the follow-up period. These 32 patients represented 72.7% (32/44) of all cases with progressive CLL or relapse. In patients with informative follow-up, emergence of the resistance mutation predated the first clinical observation of relapse or disease progression with median 9 months.

With the sensitive interrogation of *BCL2* p.Gly101Val and p.Asp103Tyr, underlying molecular mechanisms of acquired venetoclax resistance was uncovered in ten patients representing 43.5% (10/23) of all cases developing secondary resistance during the follow-up period. Interestingly, all *BCL2* p.Gly101Val and p.Asp103Tyr variants were detected in patients receiving venetoclax as a continuous single-agent treatment, while the two resistance mutations were not observed during or after fixed duration venetoclax therapy. Targeted next-generation sequencing unveiled additional co-existing *BCL2* mutations in four patients suggesting a complex underlying architecture of resistant CLL cell compartments, coupled with subsequent progressive CLL in all four cases.

In summary, our study demonstrates the value and clinical feasibility of sensitive ddPCR-based methods in the detection of underlying genetic changes in acquired ibrutinib and venetoclax resistance. With a series of experiment, we have scrutinized the correlation between the emergence of distinct resistance mutations and disease progression in R/R CLL treated with targeted agents with the ultimate goal to aid the standard-of-care monitoring of the efficacy of ibrutinib and venetoclax therapies in CLL.

VIII. REFERENCES

1. M Hallek, TD Shanafelt and B Eichhorst. Chronic lymphocytic leukaemia. Lancet. 2018;391(10129):1524.

2. A Sud, S Chattopadhyay, H Thomsen, K Sundquist, J Sundquist, RS Houlston and K Hemminki. Analysis of 153 115 patients with hematological malignancies refines the spectrum of familial risk. Blood. 2019;134(12):960.

3. SH Swerdlow, E Campo, NL Harris, ES Jaffe, SA Pileri, H Stein and J Thiele. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. FT Bosman, ES Jaffe, SR Lakhani and H Ohgaki, editors. Lyon: International Agency for Research on Cancer; 2017.

4. M Hallek, BD Cheson, D Catovsky, F Caligaris-Cappio, G Dighiero, H Dohner, P Hillmen, M Keating, E Montserrat, N Chiorazzi, S Stilgenbauer, KR Rai, JC Byrd, B Eichhorst, S O'Brien, T Robak, JF Seymour and TJ Kipps. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018;131(25):2745.

5. AC Rawstron, KA Kreuzer, A Soosapilla, M Spacek, O Stehlikova, P Gambell, N McIver-Brown, N Villamor, K Psarra, M Arroz, R Milani, J de la Serna, MT Cedena, O Jaksic, J Nomdedeu, C Moreno, GM Rigolin, A Cuneo, P Johansen, HE Johnsen, R Rosenquist, CU Niemann, W Kern, D Westerman, M Trneny, S Mulligan, M Doubek, S Pospisilova, P Hillmen, D Oscier, M Hallek, P Ghia and E Montserrat. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry B Clin Cytom. 2018;94(1):121.

6. RN Damle, T Wasil, F Fais, F Ghiotto, A Valetto, SL Allen, A Buchbinder, D Budman, K Dittmar, J Kolitz, SM Lichtman, P Schulman, VP Vinciguerra, KR Rai, M Ferrarini and N Chiorazzi. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94(6):1840.

7. H Dohner, S Stilgenbauer, A Benner, E Leupolt, A Krober, L Bullinger, K Dohner, M Bentz and P Lichter. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26):1910.

8. M Hallek, K Fischer, G Fingerle-Rowson, AM Fink, R Busch, J Mayer, M Hensel, G Hopfinger, G Hess, U von Grunhagen, M Bergmann, J Catalano, PL Zinzani, F

Caligaris-Cappio, JF Seymour, A Berrebi, U Jager, B Cazin, M Trneny, A Westermann, CM Wendtner, BF Eichhorst, P Staib, A Buhler, D Winkler, T Zenz, S Bottcher, M Ritgen, M Mendila, M Kneba, H Dohner, S Stilgenbauer, I International Group of and G German Chronic Lymphocytic Leukaemia Study. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010;376(9747):1164.

9. T Zenz, D Vollmer, M Trbusek, J Smardova, A Benner, T Soussi, H Helfrich, M Heuberger, P Hoth, M Fuge, T Denzel, S Habe, J Malcikova, P Kuglik, S Truong, N Patten, L Wu, D Oscier, R Ibbotson, A Gardiner, I Tracy, K Lin, A Pettitt, S Pospisilova, J Mayer, M Hallek, H Dohner, S Stilgenbauer and CLL European Research Initiative on. TP53 mutation profile in chronic lymphocytic leukemia: evidence for a disease specific profile from a comprehensive analysis of 268 mutations. Leukemia. 2010;24(12):2072.

10. M Hallek and O Al-Sawaf. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol. 2021;96(12):1679.

11. H Dohner, S Stilgenbauer, MR James, A Benner, T Weilguni, M Bentz, K Fischer, W Hunstein and P Lichter. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood. 1997;89(7):2516.

12. Y Le Bris, S Struski, R Guieze, C Rouvellat, N Prade, X Troussard, O Tournilhac, MC Bene, E Delabesse and L Ysebaert. Major prognostic value of complex karyotype in addition to TP53 and IGHV mutational status in first-line chronic lymphocytic leukemia. Hematol Oncol. 2017;35(4):664.

13. GM Rigolin, M Cavallari, FM Quaglia, L Formigaro, E Lista, A Urso, E Guardalben, C Liberatore, D Faraci, E Saccenti, C Bassi, L Lupini, MA Bardi, E Volta, E Tammiso, A Melandri, M Negrini, F Cavazzini and A Cuneo. In CLL, comorbidities and the complex karyotype are associated with an inferior outcome independently of CLL-IPI. Blood. 2017;129(26):3495.

14. F Nadeu, J Delgado, C Royo, T Baumann, T Stankovic, M Pinyol, P Jares, A Navarro, D Martin-Garcia, S Bea, I Salaverria, C Oldreive, M Aymerich, H Suarez-Cisneros, M Rozman, N Villamor, D Colomer, A Lopez-Guillermo, M Gonzalez, M Alcoceba, MJ Terol, E Colado, XS Puente, C Lopez-Otin, A Enjuanes and E Campo.

Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood. 2016;127(17):2122.

15. XS Puente, M Pinyol, V Quesada, L Conde, GR Ordonez, N Villamor, G Escaramis, P Jares, S Bea, M Gonzalez-Diaz, L Bassaganyas, T Baumann, M Juan, M Lopez-Guerra, D Colomer, JM Tubio, C Lopez, A Navarro, C Tornador, M Aymerich, M Rozman, JM Hernandez, DA Puente, JM Freije, G Velasco, A Gutierrez-Fernandez, D Costa, A Carrio, S Guijarro, A Enjuanes, L Hernandez, J Yague, P Nicolas, CM Romeo-Casabona, H Himmelbauer, E Castillo, JC Dohm, S de Sanjose, MA Piris, E de Alava, J San Miguel, R Royo, JL Gelpi, D Torrents, M Orozco, DG Pisano, A Valencia, R Guigo, M Bayes, S Heath, M Gut, P Klatt, J Marshall, K Raine, LA Stebbings, PA Futreal, MR Stratton, PJ Campbell, I Gut, A Lopez-Guillermo, X Estivill, E Montserrat, C Lopez-Otin and E Campo. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011;475(7354):101.

16. V Quesada, L Conde, N Villamor, GR Ordonez, P Jares, L Bassaganyas, AJ Ramsay, S Bea, M Pinyol, A Martinez-Trillos, M Lopez-Guerra, D Colomer, A Navarro, T Baumann, M Aymerich, M Rozman, J Delgado, E Gine, JM Hernandez, M Gonzalez-Diaz, DA Puente, G Velasco, JM Freije, JM Tubio, R Royo, JL Gelpi, M Orozco, DG Pisano, J Zamora, M Vazquez, A Valencia, H Himmelbauer, M Bayes, S Heath, M Gut, I Gut, X Estivill, A Lopez-Guillermo, XS Puente, E Campo and C Lopez-Otin. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet. 2011;44(1):47.

17. E Campo, F Cymbalista, P Ghia, U Jager, S Pospisilova, R Rosenquist, A Schuh and S Stilgenbauer. TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. Haematologica. 2018;103(12):1956.

18. TJ Hamblin, Z Davis, A Gardiner, DG Oscier and FK Stevenson. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94(6):1848.

19. R Rosenquist, P Ghia, A Hadzidimitriou, LA Sutton, A Agathangelidis, P Baliakas, N Darzentas, V Giudicelli, MP Lefranc, AW Langerak, C Belessi, F Davi and K Stamatopoulos. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. Leukemia. 2017;31(7):1477.

20. K Stamatopoulos, C Belessi, C Moreno, M Boudjograh, G Guida, T Smilevska, L Belhoul, S Stella, N Stavroyianni, M Crespo, A Hadzidimitriou, L Sutton, F Bosch, N Laoutaris, A Anagnostopoulos, E Montserrat, A Fassas, G Dighiero, F Caligaris-Cappio, H Merle-Beral, P Ghia and F Davi. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: Pathogenetic implications and clinical correlations. Blood. 2007;109(1):259.

21. P Baliakas, A Hadzidimitriou, LA Sutton, E Minga, A Agathangelidis, M Nichelatti, A Tsanousa, L Scarfo, Z Davis, XJ Yan, T Shanafelt, K Plevova, Y Sandberg, FJ Vojdeman, M Boudjogra, T Tzenou, M Chatzouli, CC Chu, S Veronese, A Gardiner, L Mansouri, KE Smedby, LB Pedersen, K van Lom, V Giudicelli, HS Francova, F Nguyen-Khac, P Panagiotidis, G Juliusson, L Angelis, A Anagnostopoulos, MP Lefranc, M Facco, L Trentin, M Catherwood, M Montillo, CH Geisler, AW Langerak, S Pospisilova, N Chiorazzi, D Oscier, DF Jelinek, N Darzentas, C Belessi, F Davi, R Rosenquist, P Ghia and K Stamatopoulos. Clinical effect of stereotyped B-cell receptor immunoglobulins in chronic lymphocytic leukaemia: a retrospective multicentre study. Lancet Haematol. 2014;1(2):e74.

22. LA Sutton, E Young, P Baliakas, A Hadzidimitriou, T Moysiadis, K Plevova, D Rossi, J Kminkova, E Stalika, LB Pedersen, J Malcikova, A Agathangelidis, Z Davis, L Mansouri, L Scarfo, M Boudjoghra, A Navarro, AF Muggen, XJ Yan, F Nguyen-Khac, M Larrayoz, P Panagiotidis, N Chiorazzi, CU Niemann, C Belessi, E Campo, JC Strefford, AW Langerak, D Oscier, G Gaidano, S Pospisilova, F Davi, P Ghia, K Stamatopoulos, R Rosenquist and tERIoCLL Eric. Different spectra of recurrent gene mutations in subsets of chronic lymphocytic leukemia harboring stereotyped B-cell receptors. Haematologica. 2016;101(8):959.

23. KR Rai, A Sawitsky, EP Cronkite, AD Chanana, RN Levy and BS Pasternack. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46(2):219.

24. JL Binet, A Auquier, G Dighiero, C Chastang, H Piguet, J Goasguen, G Vaugier, G Potron, P Colona, F Oberling, M Thomas, G Tchernia, C Jacquillat, P Boivin, C Lesty, MT Duault, M Monconduit, S Belabbes and F Gremy. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48(1):198.

25. N Pflug, J Bahlo, TD Shanafelt, BF Eichhorst, MA Bergmann, T Elter, K Bauer, G Malchau, KG Rabe, S Stilgenbauer, H Dohner, U Jager, MJ Eckart, G Hopfinger, R Busch, AM Fink, CM Wendtner, K Fischer, NE Kay and M Hallek. Development of a comprehensive prognostic index for patients with chronic lymphocytic leukemia. Blood. 2014;124(1):49.

26. CLLIPIwg International. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016;17(6):779.

27. C Shustik, R Mick, R Silver, A Sawitsky, K Rai and L Shapiro. Treatment of early chronic lymphocytic leukemia: intermittent chlorambucil versus observation. Hematol Oncol. 1988;6(1):7.

28. BF Eichhorst, R Busch, G Hopfinger, R Pasold, M Hensel, C Steinbrecher, S Siehl, U Jager, M Bergmann, S Stilgenbauer, C Schweighofer, CM Wendtner, H Dohner, G Brittinger, B Emmerich, M Hallek and CLLSG German. Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood. 2006;107(3):885.

29. FK Stevenson, S Krysov, AJ Davies, AJ Steele and G Packham. B-cell receptor signaling in chronic lymphocytic leukemia. Blood. 2011;118(16):4313.

30. JR Brown, JC Byrd, SE Coutre, DM Benson, IW Flinn, ND Wagner-Johnston, SE Spurgeon, BS Kahl, C Bello, HK Webb, DM Johnson, S Peterman, D Li, TM Jahn, BJ Lannutti, RG Ulrich, AS Yu, LL Miller and RR Furman. Idelalisib, an inhibitor of phosphatidylinositol 3-kinase p110delta, for relapsed/refractory chronic lymphocytic leukemia. Blood. 2014;123(22):3390.

31. IW Flinn, S O'Brien, B Kahl, M Patel, Y Oki, FF Foss, P Porcu, J Jones, JA Burger, N Jain, VM Kelly, K Allen, M Douglas, J Sweeney, P Kelly and S Horwitz. Duvelisib, a novel oral dual inhibitor of PI3K-delta,gamma, is clinically active in advanced hematologic malignancies. Blood. 2018;131(8):877.

32. KA Kreuzer, RR Furman, S Stilgenbauer, RL Dubowy, Y Kim, V Munugalavadla, E Lilienweiss, HC Reinhardt, P Cramer, B Eichhorst, P Hillmen, SM O'Brien, AR Pettitt and M Hallek. The impact of complex karyotype on the overall survival of patients with relapsed chronic lymphocytic leukemia treated with idelalisib plus rituximab. Leukemia. 2020;34(1):296.

33. SE Herman, AL Gordon, E Hertlein, A Ramanunni, X Zhang, S Jaglowski, J Flynn, J Jones, KA Blum, JJ Buggy, A Hamdy, AJ Johnson and JC Byrd. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. Blood. 2011;117(23):6287.

34. JA Burger, A Tedeschi, PM Barr, T Robak, C Owen, P Ghia, O Bairey, P Hillmen, NL Bartlett, J Li, D Simpson, S Grosicki, S Devereux, H McCarthy, S Coutre, H Quach, G Gaidano, Z Maslyak, DA Stevens, A Janssens, F Offner, J Mayer, M O'Dwyer, A Hellmann, A Schuh, T Siddiqi, A Polliack, CS Tam, D Suri, M Cheng, F Clow, L Styles, DF James, TJ Kipps and R- Investigators. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. N Engl J Med. 2015;373(25):2425.

35. JC Byrd, RR Furman, SE Coutre, IW Flinn, JA Burger, KA Blum, B Grant, JP Sharman, M Coleman, WG Wierda, JA Jones, W Zhao, NA Heerema, AJ Johnson, J Sukbuntherng, BY Chang, F Clow, E Hedrick, JJ Buggy, DF James and S O'Brien. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013;369(1):32.

36. JC Byrd, JR Brown, S O'Brien, JC Barrientos, NE Kay, NM Reddy, S Coutre, CS Tam, SP Mulligan, U Jaeger, S Devereux, PM Barr, RR Furman, TJ Kipps, F Cymbalista, C Pocock, P Thornton, F Caligaris-Cappio, T Robak, J Delgado, SJ Schuster, M Montillo, A Schuh, S de Vos, D Gill, A Bloor, C Dearden, C Moreno, JJ Jones, AD Chu, M Fardis, J McGreivy, F Clow, DF James, P Hillmen and R Investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med. 2014;371(3):213.

37. JC Byrd, RR Furman, SE Coutre, IW Flinn, JA Burger, K Blum, JP Sharman, W Wierda, W Zhao, NA Heerema, Y Luan, EA Liu, JP Dean and S O'Brien. Ibrutinib Treatment for First-Line and Relapsed/Refractory Chronic Lymphocytic Leukemia: Final Analysis of the Pivotal Phase Ib/II PCYC-1102 Study. Clin Cancer Res. 2020;26(15):3918.

38. K Aarup, EC Rotbain, L Enggaard, RS Pedersen, OJ Bergmann, RH Thomsen, M Frederiksen, H Frederiksen, T Nielsen, I Christiansen, MA Andersen and CU Niemann. Real-world outcomes for 205 patients with chronic lymphocytic leukemia treated with ibrutinib. Eur J Haematol. 2020;105(5):646.

39. M Winqvist, PO Andersson, A Asklid, K Karlsson, C Karlsson, B Lauri, J Lundin, M Mattsson, S Norin, A Sandstedt, R Rosenquist, F Spath, L Hansson, A Osterborg and CLLG Swedish. Long-term real-world results of ibrutinib therapy in patients with relapsed or refractory chronic lymphocytic leukemia: 30-month follow up of the Swedish compassionate use cohort. Haematologica. 2019;104(5):e208.

40. JR Brown, P Hillmen, S O'Brien, JC Barrientos, NM Reddy, SE Coutre, CS Tam, SP Mulligan, U Jaeger, PM Barr, RR Furman, TJ Kipps, F Cymbalista, P Thornton, F Caligaris-Cappio, J Delgado, M Montillo, S DeVos, C Moreno, JM Pagel, T Munir, JA Burger, D Chung, J Lin, L Gau, B Chang, G Cole, E Hsu, DF James and JC Byrd. Extended follow-up and impact of high-risk prognostic factors from the phase 3 RESONATE study in patients with previously treated CLL/SLL. Leukemia. 2018;32(1):83.

41. S O'Brien, JA Jones, SE Coutre, AR Mato, P Hillmen, C Tam, A Osterborg, T Siddiqi, MJ Thirman, RR Furman, O Ilhan, MJ Keating, TG Call, JR Brown, M Stevens-Brogan, Y Li, F Clow, DF James, AD Chu, M Hallek and S Stilgenbauer. Ibrutinib for patients with relapsed or refractory chronic lymphocytic leukaemia with 17p deletion (RESONATE-17): a phase 2, open-label, multicentre study. Lancet Oncol. 2016;17(10):1409.

42. G Salles, E Bachy, L Smolej, M Simkovic, L Baseggio, A Panovska, H Besson, N Healy, J Garside, W Iraqi, J Diels, C Pick-Lauer, M Spacek, R Urbanova, D Lysak, R Hermans, J Lundbom, E Callet-Bauchu and M Doubek. Single-agent ibrutinib in RESONATE-2 and RESONATE versus treatments in the real-world PHEDRA databases for patients with chronic lymphocytic leukemia. Ann Hematol. 2019;98(12):2749.

43. PA Thompson, SM O'Brien, WG Wierda, A Ferrajoli, F Stingo, SC Smith, JA Burger, Z Estrov, N Jain, HM Kantarjian and MJ Keating. Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. Cancer. 2015;121(20):3612.

44. JP Sharman, M Egyed, W Jurczak, A Skarbnik, JM Pagel, IW Flinn, M Kamdar, T Munir, R Walewska, G Corbett, LM Fogliatto, Y Herishanu, V Banerji, S Coutre, G Follows, P Walker, K Karlsson, P Ghia, A Janssens, F Cymbalista, JA Woyach, G Salles, WG Wierda, R Izumi, V Munugalavadla, P Patel, MH Wang, S Wong and JC Byrd.

Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzmab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. Lancet. 2020;395(10232):1278.

45. P Ghia, A Pluta, M Wach, D Lysak, T Kozak, M Simkovic, P Kaplan, I Kraychok, A Illes, J de la Serna, S Dolan, P Campbell, G Musuraca, A Jacob, E Avery, JH Lee, W Liang, P Patel, C Quah and W Jurczak. ASCEND: Phase III, Randomized Trial of Acalabrutinib Versus Idelalisib Plus Rituximab or Bendamustine Plus Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. J Clin Oncol. 2020;38(25):2849.

46. CS Tam, JR Brown, BS Kahl, P Ghia, K Giannopoulos, W Jurczak, M Simkovic, M Shadman, A Osterborg, L Laurenti, P Walker, S Opat, H Chan, H Ciepluch, R Greil, M Tani, M Trneny, DM Brander, IW Flinn, S Grosicki, E Verner, A Tedeschi, J Li, T Tian, L Zhou, C Marimpietri, JC Paik, A Cohen, J Huang, T Robak and P Hillmen. Zanubrutinib versus bendamustine and rituximab in untreated chronic lymphocytic leukaemia and small lymphocytic lymphoma (SEQUOIA): a randomised, controlled, phase 3 trial. Lancet Oncol. 2022;23(8):1031.

47. CS Tam, J Trotman, S Opat, JA Burger, G Cull, D Gottlieb, R Harrup, PB Johnston, P Marlton, J Munoz, JF Seymour, D Simpson, A Tedeschi, R Elstrom, Y Yu, Z Tang, L Han, J Huang, W Novotny, L Wang and AW Roberts. Phase 1 study of the selective BTK inhibitor zanubrutinib in B-cell malignancies and safety and efficacy evaluation in CLL. Blood. 2019;134(11):851.

48. P Hillmen, JR Brown, BF Eichhorst, N Lamanna, SM O'Brien, L Qiu, T Salmi, J Hilger, K Wu, A Cohen, J Huang and CS Tam. ALPINE: zanubrutinib versus ibrutinib in relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma. Future Oncol. 2020;16(10):517.

49. AR Mato, NN Shah, W Jurczak, CY Cheah, JM Pagel, JA Woyach, B Fakhri, TA Eyre, N Lamanna, MR Patel, A Alencar, E Lech-Maranda, WG Wierda, CC Coombs, JN Gerson, P Ghia, S Le Gouill, DJ Lewis, S Sundaram, JB Cohen, IW Flinn, CS Tam, MA Barve, B Kuss, J Taylor, O Abdel-Wahab, SJ Schuster, ML Palomba, KL Lewis, LE Roeker, MS Davids, XN Tan, TS Fenske, J Wallin, DE Tsai, NC Ku, E Zhu, J Chen, M Yin, B Nair, K Ebata, N Marella, JR Brown and M Wang. Pirtobrutinib in relapsed or

refractory B-cell malignancies (BRUIN): a phase 1/2 study. Lancet. 2021;397(10277):892.

50. A Gango, D Alpar, B Galik, D Marosvari, R Kiss, V Fesus, D Aczel, E Eyupoglu, N Nagy, A Nagy, S Krizsan, L Reiniger, P Farkas, A Kozma, E Adam, S Tasnady, M Reti, A Matolcsy, A Gyenesei, Z Matrai and C Bodor. Dissection of subclonal evolution by temporal mutation profiling in chronic lymphocytic leukemia patients treated with ibrutinib. Int J Cancer. 2020;146(1):85.

51. T Munir, JR Brown, S O'Brien, JC Barrientos, PM Barr, NM Reddy, S Coutre, CS Tam, SP Mulligan, U Jaeger, TJ Kipps, C Moreno, M Montillo, JA Burger, JC Byrd, P Hillmen, S Dai, A Szoke, JP Dean and JA Woyach. Final analysis from RESONATE: Up to six years of follow-up on ibrutinib in patients with previously treated chronic lymphocytic leukemia or small lymphocytic lymphoma. Am J Hematol. 2019;94(12):1353.

52. KJ Maddocks, AS Ruppert, G Lozanski, NA Heerema, W Zhao, L Abruzzo, A Lozanski, M Davis, A Gordon, LL Smith, R Mantel, JA Jones, JM Flynn, SM Jaglowski, LA Andritsos, F Awan, KA Blum, MR Grever, AJ Johnson, JC Byrd and JA Woyach. Etiology of Ibrutinib Therapy Discontinuation and Outcomes in Patients With Chronic Lymphocytic Leukemia. JAMA Oncol. 2015;1(1):80.

53. JA Woyach, AS Ruppert, D Guinn, A Lehman, JS Blachly, A Lozanski, NA Heerema, W Zhao, J Coleman, D Jones, L Abruzzo, A Gordon, R Mantel, LL Smith, S McWhorter, M Davis, TJ Doong, F Ny, M Lucas, W Chase, JA Jones, JM Flynn, K Maddocks, K Rogers, S Jaglowski, LA Andritsos, FT Awan, KA Blum, MR Grever, G Lozanski, AJ Johnson and JC Byrd. BTK(C481S)-Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia. J Clin Oncol. 2017;35(13):1437.

54. IE Ahn, C Underbayev, A Albitar, SE Herman, X Tian, I Maric, DC Arthur, L Wake, S Pittaluga, CM Yuan, M Stetler-Stevenson, S Soto, J Valdez, P Nierman, J Lotter, L Xi, M Raffeld, M Farooqui, M Albitar and A Wiestner. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood. 2017;129(11):1469.

55. JA Burger, DA Landau, A Taylor-Weiner, I Bozic, H Zhang, K Sarosiek, L Wang, C Stewart, J Fan, J Hoellenriegel, M Sivina, AM Dubuc, C Fraser, Y Han, S Li, KJ Livak, L Zou, Y Wan, S Konoplev, C Sougnez, JR Brown, LV Abruzzo, SL Carter, MJ Keating, MS Davids, WG Wierda, K Cibulskis, T Zenz, L Werner, P Dal Cin, P Kharchencko, D

Neuberg, H Kantarjian, E Lander, S Gabriel, S O'Brien, A Letai, DA Weitz, MA Nowak, G Getz and CJ Wu. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. Nat Commun. 2016;7:11589.

56. A Quinquenel, LM Fornecker, R Letestu, L Ysebaert, C Fleury, G Lazarian, MS Dilhuydy, D Nollet, R Guieze, P Feugier, D Roos-Weil, L Willems, AS Michallet, A Delmer, K Hormigos, V Levy, F Cymbalista, F Baran-Marszak and CLLG French Innovative Leukemia Organization. Prevalence of BTK and PLCG2 mutations in a real-life CLL cohort still on ibrutinib after 3 years: a FILO group study. Blood. 2019;134(7):641.

57. S Kadri, J Lee, C Fitzpatrick, N Galanina, M Sukhanova, G Venkataraman, S Sharma, B Long, K Petras, M Theissen, M Ming, Y Kobzev, W Kang, A Guo, W Wang, N Niu, H Weiner, M Thirman, W Stock, SM Smith, C Nabhan, JP Segal, P Lu and YL Wang. Clonal evolution underlying leukemia progression and Richter transformation in patients with ibrutinib-relapsed CLL. Blood Adv. 2017;1(12):715.

58. JA Woyach, RR Furman, TM Liu, HG Ozer, M Zapatka, AS Ruppert, L Xue, DH Li, SM Steggerda, M Versele, SS Dave, J Zhang, AS Yilmaz, SM Jaglowski, KA Blum, A Lozanski, G Lozanski, DF James, JC Barrientos, P Lichter, S Stilgenbauer, JJ Buggy, BY Chang, AJ Johnson and JC Byrd. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014;370(24):2286.

59. RR Furman, S Cheng, P Lu, M Setty, AR Perez, A Guo, J Racchumi, G Xu, H Wu, J Ma, SM Steggerda, M Coleman, C Leslie and YL Wang. Ibrutinib resistance in chronic lymphocytic leukemia. N Engl J Med. 2014;370(24):2352.

60. C Bodor, L Kotmayer, T Laszlo, F Takacs, G Barna, R Kiss, E Sebestyen, T Nagy, LL Hegyi, G Mikala, S Fekete, P Farkas, A Balogh, T Masszi, J Demeter, J Weisinger, H Alizadeh, B Kajtar, Z Kohl, R Szasz, L Gergely, T Gurbity Palfi, A Sulak, B Kollar, M Egyed, M Plander, L Rejto, L Szerafin, P Ilonczai, P Tamaska, P Pettendi, D Levai, T Schneider, A Sebestyen, P Csermely, A Matolcsy, Z Matrai and D Alpar. Screening and monitoring of the BTK(C481S) mutation in a real-world cohort of patients with relapsed/refractory chronic lymphocytic leukaemia during ibrutinib therapy. Br J Haematol. 2021;194(2):355.

61. TG Lama, D Kyung and S O'Brien. Mechanisms of ibrutinib resistance in chronic lymphocytic leukemia and alternative treatment strategies. Expert Rev Hematol. 2020;13(8):871.

62. R Kiss, D Alpar, A Gango, N Nagy, E Eyupoglu, D Aczel, A Matolcsy, J Csomor, Z Matrai and C Bodor. Spatial clonal evolution leading to ibrutinib resistance and disease progression in chronic lymphocytic leukemia. Haematologica. 2019;104(1):e38.

63. DT Chao and SJ Korsmeyer. BCL-2 family: regulators of cell death. Annu Rev Immunol. 1998;16:395.

64. AW Roberts, MS Davids, JM Pagel, BS Kahl, SD Puvvada, JF Gerecitano, TJ Kipps, MA Anderson, JR Brown, L Gressick, S Wong, M Dunbar, M Zhu, MB Desai, E Cerri, S Heitner Enschede, RA Humerickhouse, WG Wierda and JF Seymour. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med. 2016;374(4):311.

65. JF Seymour, TJ Kipps, B Eichhorst, P Hillmen, J D'Rozario, S Assouline, C Owen, J Gerecitano, T Robak, J De la Serna, U Jaeger, G Cartron, M Montillo, R Humerickhouse, EA Punnoose, Y Li, M Boyer, K Humphrey, M Mobasher and AP Kater. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. N Engl J Med. 2018;378(12):1107.

66. K Fischer, O Al-Sawaf, J Bahlo, AM Fink, M Tandon, M Dixon, S Robrecht, S Warburton, K Humphrey, O Samoylova, AM Liberati, J Pinilla-Ibarz, S Opat, L Sivcheva, K Le Du, LM Fogliatto, CU Niemann, R Weinkove, S Robinson, TJ Kipps, S Boettcher, E Tausch, R Humerickhouse, B Eichhorst, CM Wendtner, AW Langerak, KA Kreuzer, M Ritgen, V Goede, S Stilgenbauer, M Mobasher and M Hallek. Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. N Engl J Med. 2019;380(23):2225.

67. AR Mato, C Tam, J Allan, DM Brander, JM Pagel, CS Ujjani, BT Hill, N Lamanna, F Lansigan, R Jacobs, M Shadman, A Skarbnik, JJ Pu, PM Barr, AR Sehgal, BD Cheson, CS Zent, HH Tuncer, SJ Schuster, PV Pickens, NN Shah, AM Winter, K Kennard, K Isaac, C Dorsey, AM Vandegrift, AK Singavi, A Williams, C Howlett, H Weissbrot, N Ali, S Khajavian, A Sitlinger, E Tranchito, J Rhodes, E Bharat Bhavsar, N Bailey, B Patel, TF Burns, M Yacur, M Malhotra, SM Handunnetti, J Svoboda, RR Furman and C Nabhan. Disease and Patient Characteristics, Patterns of Care, Toxicities,

and Outcomes of Chronic Lymphocytic Leukemia (CLL) Patients Treated with Venetoclax: A Multicenter Study of 204 Patients. Blood. 2017;130:4315.

68. S Stilgenbauer, B Eichhorst, J Schetelig, P Hillmen, JF Seymour, S Coutre, W Jurczak, SP Mulligan, A Schuh, S Assouline, CM Wendtner, AW Roberts, MS Davids, J Bloehdorn, T Munir, S Bottcher, L Zhou, AH Salem, M Desai, B Chyla, J Arzt, SY Kim, M Verdugo, G Gordon, M Hallek and WG Wierda. Venetoclax for Patients With Chronic Lymphocytic Leukemia With 17p Deletion: Results From the Full Population of a Phase II Pivotal Trial. J Clin Oncol. 2018;36(19):1973.

69. AJ Souers, JD Leverson, ER Boghaert, SL Ackler, ND Catron, J Chen, BD Dayton, H Ding, SH Enschede, WJ Fairbrother, DC Huang, SG Hymowitz, S Jin, SL Khaw, PJ Kovar, LT Lam, J Lee, HL Maecker, KC Marsh, KD Mason, MJ Mitten, PM Nimmer, A Oleksijew, CH Park, CM Park, DC Phillips, AW Roberts, D Sampath, JF Seymour, ML Smith, GM Sullivan, SK Tahir, C Tse, MD Wendt, Y Xiao, JC Xue, H Zhang, RA Humerickhouse, SH Rosenberg and SW Elmore. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202.

70. S Stilgenbauer, B Eichhorst, J Schetelig, S Coutre, JF Seymour, T Munir, SD Puvvada, CM Wendtner, AW Roberts, W Jurczak, SP Mulligan, S Bottcher, M Mobasher, M Zhu, M Desai, B Chyla, M Verdugo, SH Enschede, E Cerri, R Humerickhouse, G Gordon, M Hallek and WG Wierda. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. Lancet Oncol. 2016;17(6):768.

71. JF Seymour, S Ma, DM Brander, MY Choi, J Barrientos, MS Davids, MA Anderson, AW Beaven, ST Rosen, CS Tam, B Prine, SK Agarwal, W Munasinghe, M Zhu, LL Lash, M Desai, E Cerri, M Verdugo, SY Kim, RA Humerickhouse, GB Gordon, TJ Kipps and AW Roberts. Venetoclax plus rituximab in relapsed or refractory chronic lymphocytic leukaemia: a phase 1b study. Lancet Oncol. 2017;18(2):230.

72. AP Kater, JQ Wu, T Kipps, B Eichhorst, P Hillmen, J D'Rozario, S Assouline, C Owen, T Robak, J de la Serna, U Jaeger, G Cartron, M Montillo, J Dubois, E Eldering, C Mellink, AM Van Der Kevie-Kersemaekers, SY Kim, B Chyla, E Punnoose, CR Bolen, ZJ Assaf, Y Jiang, J Wang, M Lefebure, M Boyer, K Humphrey and JF Seymour. Venetoclax Plus Rituximab in Relapsed Chronic Lymphocytic Leukemia: 4-Year Results

and Evaluation of Impact of Genomic Complexity and Gene Mutations From the MURANO Phase III Study. J Clin Oncol. 2020;38(34):4042.

73. AP Kater, T Kipps, B Eichhorst, P Hillmen, J D'Rozario, C Owen, S Assouline, N Lamanna, T Robak, J de la Serna, U Jaeger, G Cartron, M Montillo, C Mellink, B Chyla, C Wilson, J Wu, Y Jiang, M Lefebure, M Boyer and JF Seymour. Five-Year Analysis of Murano Study Demonstrates Enduring Undetectable Minimal Residual Disease (uMRD) in a Subset of Relapsed/Refractory Chronic Lymphocytic Leukemia (R/R CLL) Patients (Pts) Following Fixed-Duration Venetoclax-Rituximab (VenR) Therapy (Tx). Blood. 2020;136:19.

74. O Al-Sawaf, C Zhang, M Tandon, A Sinha, AM Fink, S Robrecht, O Samoylova, AM Liberati, J Pinilla-Ibarz, S Opat, L Sivcheva, K Le Du, LM Fogliatto, CU Niemann, R Weinkove, S Robinson, TJ Kipps, E Tausch, W Schary, M Ritgen, CM Wendtner, KA Kreuzer, B Eichhorst, S Stilgenbauer, M Hallek and K Fischer. Venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab for previously untreated chronic lymphocytic leukaemia (CLL14): follow-up results from a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2020;21(9):1188.

75. GP Sullivan, L Flanagan, DA Rodrigues and T Ni Chonghaile. The path to venetoclax resistance is paved with mutations, metabolism, and more. Sci Transl Med. 2022;14(674):eabo6891.

76. P Blombery, MA Anderson, JN Gong, R Thijssen, RW Birkinshaw, ER Thompson, CE Teh, T Nguyen, Z Xu, C Flensburg, TE Lew, IJ Majewski, DHD Gray, DA Westerman, CS Tam, JF Seymour, PE Czabotar, DCS Huang and AW Roberts. Acquisition of the Recurrent Gly101Val Mutation in BCL2 Confers Resistance to Venetoclax in Patients with Progressive Chronic Lymphocytic Leukemia. Cancer Discov. 2019;9(3):342.

77. E Tausch, W Close, A Dolnik, J Bloehdorn, B Chyla, L Bullinger, H Dohner, D Mertens and S Stilgenbauer. Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia. Haematologica. 2019;104(9):e434.

78. P Blombery, E Thompson, T Nguyen, X Chen, M McBean, RW Birkinshaw, PE Czabotar, R Thijssen, MA Anderson, JF Seymour, DA Westerman, DCS Huang and AW Roberts. Detection of Multiple Recurrent Novel BCL2 Mutations Co-Occurring with

BCL2 Gly101Val in Patients with Chronic Lymphocytic Leukemia on Long Term Venetoclax. Blood. 2019;134:171.

79. P Blombery, ER Thompson, T Nguyen, RW Birkinshaw, JN Gong, X Chen, M McBean, R Thijssen, T Conway, MA Anderson, JF Seymour, DA Westerman, PE Czabotar, DCS Huang and AW Roberts. Multiple BCL2 mutations cooccurring with Gly101Val emerge in chronic lymphocytic leukemia progression on venetoclax. Blood. 2020;135(10):773.

80. F Lucas, K Larkin, CT Gregory, S Orwick, TJ Doong, A Lozanski, G Lozanski, S Misra, A Ngankeu, HG Ozer, D Sampath, S Thangavadivel, SA Yilmaz, KA Rogers, JC Byrd, JA Woyach and JS Blachly. Novel BCL2 mutations in venetoclax-resistant, ibrutinib-resistant CLL patients with BTK/PLCG2 mutations. Blood. 2020;135(24):2192.

81. P Blombery. Mechanisms of intrinsic and acquired resistance to venetoclax in B-cell lymphoproliferative disease. Leuk Lymphoma. 2020;61(2):257.

82. R Thijssen, L Tian, MA Anderson, C Flensburg, A Jarratt, AL Garnham, JS Jabbari, H Peng, TE Lew, CE Teh, Q Gouil, A Georgiou, T Tan, TM Djajawi, CS Tam, JF Seymour, P Blombery, DHD Gray, IJ Majewski, ME Ritchie, AW Roberts and DCS Huang. Single-cell multiomics reveal the scale of multi-layered adaptations enabling CLL relapse during venetoclax therapy. Blood. 2022.

83. ER Thompson, T Nguyen, Y Kankanige, JF Markham, MA Anderson, SM Handunnetti, R Thijssen, PS Yeh, CS Tam, JF Seymour, AW Roberts, DA Westerman and P Blombery. Single-cell sequencing demonstrates complex resistance landscape in CLL and MCL treated with BTK and BCL2 inhibitors. Blood Adv. 2022;6(2):503.

84. MA Anderson, C Tam, TE Lew, S Juneja, M Juneja, D Westerman, M Wall, S Lade, A Gorelik, DCS Huang, JF Seymour and AW Roberts. Clinicopathological features and outcomes of progression of CLL on the BCL2 inhibitor venetoclax. Blood. 2017;129(25):3362.

85. SK Tahir, ML Smith, P Hessler, LR Rapp, KB Idler, CH Park, JD Leverson and LT Lam. Potential mechanisms of resistance to venetoclax and strategies to circumvent it. BMC Cancer. 2017;17(1):399.

86. K Bojarczuk, BK Sasi, S Gobessi, I Innocenti, G Pozzato, L Laurenti and DG Efremov. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. Blood. 2016;127(25):3192.

87. D Thomalla, L Beckmann, C Grimm, M Oliverio, L Meder, CD Herling, P Nieper, T Feldmann, O Merkel, E Lorsy, A da Palma Guerreiro, J von Jan, I Kisis, E Wasserburger, J Claasen, E Faitschuk-Meyer, J Altmuller, P Nurnberg, TP Yang, M Lienhard, R Herwig, KA Kreuzer, CP Pallasch, R Buettner, SC Schafer, J Hartley, H Abken, M Peifer, H Kashkar, G Knittel, B Eichhorst, RT Ullrich, M Herling, HC Reinhardt, M Hallek, MR Schweiger and LP Frenzel. Deregulation and epigenetic modification of BCL2-family genes cause resistance to venetoclax in hematologic malignancies. Blood. 2022.

88. CD Herling, N Abedpour, J Weiss, A Schmitt, RD Jachimowicz, O Merkel, M Cartolano, S Oberbeck, P Mayer, V Berg, D Thomalla, N Kutsch, M Stiefelhagen, P Cramer, CM Wendtner, T Persigehl, A Saleh, J Altmuller, P Nurnberg, C Pallasch, V Achter, U Lang, B Eichhorst, R Castiglione, SC Schafer, R Buttner, KA Kreuzer, HC Reinhardt, M Hallek, LP Frenzel and M Peifer. Clonal dynamics towards the development of venetoclax resistance in chronic lymphocytic leukemia. Nat Commun. 2018;9(1):727.

89. R Bennett, E Thompson and C Tam. Mechanisms of Resistance to BCL2 Inhibitor Therapy in Chronic Lymphocytic Leukaemia and Potential Future Therapeutic Directions. Clin Lymphoma Myeloma Leuk. 2022;S2152-2650(22).

90. R Guieze, VM Liu, D Rosebrock, AA Jourdain, M Hernandez-Sanchez, A Martinez Zurita, J Sun, E Ten Hacken, K Baranowski, PA Thompson, JM Heo, Z Cartun, O Aygun, JB Iorgulescu, W Zhang, G Notarangelo, D Livitz, S Li, MS Davids, A Biran, SM Fernandes, JR Brown, A Lako, ZB Ciantra, MA Lawlor, DB Keskin, ND Udeshi, WG Wierda, KJ Livak, AG Letai, D Neuberg, JW Harper, SA Carr, F Piccioni, CJ Ott, I Leshchiner, CM Johannessen, J Doench, VK Mootha, G Getz and CJ Wu. Mitochondrial Reprogramming Underlies Resistance to BCL-2 Inhibition in Lymphoid Malignancies. Cancer Cell. 2019;36(4):369.

91. JA Jones, AR Mato, WG Wierda, MS Davids, M Choi, BD Cheson, RR Furman, N Lamanna, PM Barr, L Zhou, B Chyla, AH Salem, M Verdugo, RA Humerickhouse, J Potluri, S Coutre, J Woyach and JC Byrd. Venetoclax for chronic lymphocytic leukaemia progressing after ibrutinib: an interim analysis of a multicentre, open-label, phase 2 trial. Lancet Oncol. 2018;19(1):65.

92. AR Mato, M Thompson, JN Allan, DM Brander, JM Pagel, CS Ujjani, BT Hill, N Lamanna, F Lansigan, R Jacobs, M Shadman, AP Skarbnik, JJ Pu, PM Barr, AR Sehgal, BD Cheson, CS Zent, HH Tuncer, SJ Schuster, PV Pickens, NN Shah, A Goy, AM Winter, C Garcia, K Kennard, K Isaac, C Dorsey, LM Gashonia, AK Singavi, LE Roeker, A Zelenetz, A Williams, C Howlett, H Weissbrot, N Ali, S Khajavian, A Sitlinger, E Tranchito, J Rhodes, J Felsenfeld, N Bailey, B Patel, TF Burns, M Yacur, M Malhotra, J Svoboda, RR Furman and C Nabhan. Real-world outcomes and management strategies for venetoclax-treated chronic lymphocytic leukemia patients in the United States. Haematologica. 2018;103(9):1511.

93. SD Reiff, EM Muhowski, D Guinn, A Lehman, CA Fabian, C Cheney, R Mantel, L Smith, AJ Johnson, WB Young, AR Johnson, L Liu, JC Byrd and JA Woyach. Noncovalent inhibition of C481S Bruton tyrosine kinase by GDC-0853: a new treatment strategy for ibrutinib-resistant CLL. Blood. 2018;132(10):1039.

94. A Matio, I Flinn, J Pagel, J Brown, C Cheah, C Coombs, M Patel, S Rothenberg, D Tsai, N Ku and M Wang. Results from a first-in-human, proof-of-concept phase I trial in pretreated B-cell malignancies for LOXO-305, a next-generation, highly selective, noncovalent BTK inhibitor [abstract]. Blood. 2019;134 (Supplement1):501.

95. J Allan, P Patel, A Mato, W Wierda, J Ibarz, M Choi, S O'Brien, J Sharman, M Shadman, D Gladstone, M Davids, J Pagel, R Ward, G Acton, P Taverna, J Fox, R Furman and J Brown. Ongoing results of a phase 1b/2 dose-escalation and cohort-expansion study of the selective, noncovalent, reversible Bruton's tyrosine kinase inhibitor, vecabrutinib, in B-Cell malignancies [abstract]. Blood. 2019;134 (Supplement 1):3041.

96. J Woyach, D Stephens, I Flinn, S Bhat, R Savage, F Chai, S Eathiraj, L Granlund, L Szuszkiewicz, B Schwatrz and J Byrd. Final Results of Phase 1, Dose escalation study evaluating ARQ 531 in patients with relapsed or refractory B-Cell lymphoid malignancies [abstract]. Blood. 2019;134 (Supplement 1):4298.

97. AR Mato, LE Roeker, R Jacobs, BT Hill, N Lamanna, D Brander, M Shadman, CS Ujjani, MS Yazdy, GF Perini, JA Pinilla-Ibarz, J Barrientos, AP Skarbnik, P Torka, JJ Pu, JM Pagel, S Gohil, B Fakhri, M Choi, CC Coombs, J Rhodes, PM Barr, CA Portell, H Parry, CA Garcia, KJ Whitaker, AM Winter, A Sitlinger, S Khajavian, AF Grajales-Cruz, KM Isaac, P Shah, OS Akhtar, R Pocock, K Lam, TJ Voorhees, SJ Schuster, TD

Rodgers, CP Fox, N Martinez-Calle, T Munir, EB Bhavsar, N Bailey, JC Lee, HB Weissbrot, C Nabhan, JM Goodfriend, AC King, AD Zelenetz, C Dorsey, K Bigelow, BD Cheson, JN Allan and TA Eyre. Assessment of the Efficacy of Therapies Following Venetoclax Discontinuation in CLL Reveals BTK Inhibition as an Effective Strategy. Clin Cancer Res. 2020;26(14):3589.

98. VS Lin, TE Lew, SM Handunnetti, P Blombery, T Nguyen, DA Westerman, BJ Kuss, CS Tam, AW Roberts, JF Seymour and MA Anderson. BTK inhibitor therapy is effective in patients with CLL resistant to venetoclax. Blood. 2020;135(25):2266.

99. JR Brown, MS Davids, JT Chang, S Ma, J Biondo, M Mobasher, Y Mun and AR Mato. Outcomes of Ibrutinib Therapy Given after Prior Venetoclax Therapy in Ibrutinib-Naïve Patients with Relapsed/Refractory (R/R) Chronic Lymphocytic Leukemia (CLL). Blood. 2018;132:5556.

100. R Greil, G Fraser, B Leber, B Marks, G Quaresmini, J Moritz Midekke, G Semenzato, W Schary, M Boyer, M Breuleux, N Crompton, K Humphrey and P Marlton. Efficacy and Safety of Ibrutinib (IBR) after Venetoclax (VEN) Treatment in IBR-Naïve Patients with Relapsed/Refractory (R/R) Chronic Lymphocytic Leukemia (CLL): Follow-up of Patients from the MURANO Study. Blood. 2018;132:5548.

101. TE Lew, VS Lin, ER Cliff, P Blombery, ER Thompson, SM Handunnetti, DA Westerman, BJ Kuss, CS Tam, DCS Huang, JF Seymour, AW Roberts and MA Anderson. Outcomes of patients with CLL sequentially resistant to both BCL2 and BTK inhibition. Blood Adv. 2021;5(20):4054.

102. L Scarfo, S Heltai, E Albi, E Scarano, L Schiattone, L Farina, R Moia, M Deodato, A Ferrario, M Motta, G Reda, R Sancetta, M Coscia, P Rivela, L Laurenti, M Varettoni, E Perotta, A Capasso, P Ranghetti, M Colia and P Ghia. Minimal Residual Disease-Driven Treatment Intensification with Sequential Addition of Ibrutinib to Venetoclax in R/R Cll. Blood. 2022.

103. RA Jackson, VM Smith, S Jayne, C Drewes, S Bens, R Siebert, MJS Dyer and HS Walter. Successful Retreatment With Venetoclax in a Patient With Chronic Lymphocytic Leukemia. Hemasphere. 2022;6(8):e752.

104. P Hillmen, AC Rawstron, K Brock, S Munoz-Vicente, FJ Yates, R Bishop, R Boucher, D MacDonald, C Fegan, A McCaig, A Schuh, A Pettitt, JG Gribben, PEM Patten, S Devereux, A Bloor, CP Fox, F Forconi and T Munir. Ibrutinib Plus Venetoclax

in Relapsed/Refractory Chronic Lymphocytic Leukemia: The CLARITY Study. J Clin Oncol. 2019;37(30):2722.

105. CU Niemann, MD Levin, J Dubois, S Kersting, L Enggaard, GJ Veldhuis, R Mous, CHM Mellink, JA Dobber, CB Poulsen, H Frederiksen, A Janssens, I Schjodt, EC Dompeling, J Ranti, M Mattsson, M Bellido, HTT Tran, K Nasserinejad and AP Kater. Venetoclax and ibrutinib for patients with relapsed/refractory chronic lymphocytic leukemia. Blood. 2021;137(8):1117.

106. L Kotmayer, T Laszlo, G Mikala, R Kiss, L Levay, LL Hegyi, S Grof, T Nagy, G Barna, P Farkas, J Weisinger, Z Nagy, A Balogh, T Masszi, J Demeter, A Sulak, Z Kohl, H Alizadeh, M Egyed, P Pettendi, L Gergely, M Plander, Z Pauker, A Masszi, A Matolcsy, R Szasz, C Bodor and D Alpar. Landscape of BCL2 Resistance Mutations in a Real-World Cohort of Patients with Relapsed/Refractory Chronic Lymphocytic Leukemia Treated with Venetoclax. Int J Mol Sci. 2023;24(5802).

107. AC Rawstron, C Fazi, A Agathangelidis, N Villamor, R Letestu, J Nomdedeu, C Palacio, O Stehlikova, KA Kreuzer, S Liptrot, D O'Brien, RM de Tute, I Marinov, M Hauwel, M Spacek, J Dobber, AP Kater, P Gambell, A Soosapilla, G Lozanski, G Brachtl, K Lin, J Boysen, C Hanson, JL Jorgensen, M Stetler-Stevenson, C Yuan, HE Broome, L Rassenti, F Craig, J Delgado, C Moreno, F Bosch, A Egle, M Doubek, S Pospisilova, S Mulligan, D Westerman, CM Sanders, R Emerson, HS Robins, I Kirsch, T Shanafelt, A Pettitt, TJ Kipps, WG Wierda, F Cymbalista, M Hallek, P Hillmen, E Montserrat and P Ghia. A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. Leukemia. 2016;30(4):929.

108. J Malcikova, E Tausch, D Rossi, LA Sutton, T Soussi, T Zenz, AP Kater, CU Niemann, D Gonzalez, F Davi, M Gonzalez Diaz, C Moreno, G Gaidano, K Stamatopoulos, R Rosenquist, S Stilgenbauer, P Ghia, S Pospisilova and TPn European Research Initiative on Chronic Lymphocytic Leukemia. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. Leukemia. 2018;32(5):1070.

109. C Xu, X Gu, R Padmanabhan, Z Wu, Q Peng, J DiCarlo and Y Wang. smCounter2: an accurate low-frequency variant caller for targeted sequencing data with unique molecular identifiers. Bioinformatics. 2019;35(8):1299.

110. T Tikkanen, B Leroy, JL Fournier, RA Risques, J Malcikova and T Soussi. Seshat: A Web service for accurate annotation, validation, and analysis of TP53 variants generated by conventional and next-generation sequencing. Hum Mutat. 2018;39(7):925.

111. L Bouaoun, D Sonkin, M Ardin, M Hollstein, G Byrnes, J Zavadil and M Olivier. TP53 Variations in Human Cancers: New Lessons from the IARC TP53 Database and Genomics Data. Hum Mutat. 2016;37(9):865.

112. L Sedlarikova, A Petrackova, T Papajik, P Turcsanyi and E Kriegova. Resistance-Associated Mutations in Chronic Lymphocytic Leukemia Patients Treated With Novel Agents. Front Oncol. 2020;10:894.

113. B Chyla, R Popovic, C Lu, F Dunbar, D Quarless, K Robinson, S Warder, A Jacobson, L Zhou, A Souers, J Waring, A Bhathena, JD Leverson and SY Kim. Identification of Recurrent Genomic Alterations in the Apoptotic Machinery in CLL Patients Treated with Venetoclax Monotherapy. Blood. 2019;134:172.

114. BL Lampson and JR Brown. Are BTK and PLCG2 mutations necessary and sufficient for ibrutinib resistance in chronic lymphocytic leukemia? Expert Rev Hematol. 2018;11(3):185.

DOI:10.14753/SE.2023.2857

IX. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

Publications related to the PhD thesis:

1. Csaba Bödör*, Lili Kotmayer*, Tamás László, Ferenc Takács, Gábor Barna, Richárd Kiss, Endre Sebestyén, Tibor Nagy, Lajos László Hegyi, Gábor Mikala, Sándor Fekete, Péter Farkas, Alexandra Balogh, Tamás Masszi, Judit Demeter, Júlia Weisinger, Hussain Alizadeh, Béla Kajtár, Zoltán Kohl, Róbert Szász, Lajos Gergely, Timea Gurbity Pálfi, Adrienn Sulák, Balázs Kollár, Miklós Egyed, Márk Plander, László Rejtő, László Szerafin, Péter Ilonczai, Péter Tamáska, Piroska Pettendi, Dóra Lévai, Tamás Schneider, Anna Sebestyén, Péter Csermely, András Matolcsy, Zoltán Mátrai, Donát Alpár, *Screening and monitoring of BTK C481S mutation in patients with relapsed/refractory chronic lymphocytic leukemia during ibrutinib therapy*, 2021, British Journal of Haematology, 194(2), pp. 355-364
   *Joint first authors

2. Lili Kotmayer, Tamás László, Gábor Mikala, Richárd Kiss, Luca Lévay, Lajos László Hegyi, Stefánia Gróf, Gábor Barna, Péter Farkas, Júlia Weisinger, Zsolt Nagy, Alexandra Balogh, Tamás Masszi, Judit Demeter, Adrienn Sulák, Zoltán Kohl, Hussain Alizadeh, Miklós Egyed, Piroska Pettendi, Lajos Gergely, Márk Plander, Zsolt Pauker, András Masszi, András Matolcsy, Róbert Szász, Csaba Bödör,* Donát Alpár*, *Landscape of BCL2 resistance mutations in a real-world cohort of patients with relapsed/refractory chronic lymphocytic leukemia treated with venetoclax*, 2023, International Journal of Molecular Sciences, 24(6), 5802
   *Joint senior authors

Other publications:

1. Lili Kotmayer, Damia Romero-Moya, Oskar Marin-Bejar, Emilia Kozyra, Albert Català, Anna Bigas, Marcin W Wlodarski, Csaba Bödör, Alessandra Giorgetti, *GATA2 Deficiency and MDS/AML: Experimental Strategies for Disease*

Modelling and Future Therapeutic Prospects, 2022, British Journal of Haematology, 199(4), pp. 482-495

2. Lili Kotmayer, Csaba Bödör, *[The role of next-generation sequencing in clinical oncology],* 2021, Klinikai Onkológia, 8(2), pp. 103-114 (Hungarian)

3. Lili Kotmayer, Gergő Papp, Kornélia Baghy, Erik Zajta, András Matolcsy, Csaba Bödör, *[Routine application of molecular diagnostic techniques for precision diagnostics and therapy in oncology]*, 2021, Orvosképzés, 46(3), pp. 311-321 (Hungarian)

4. Lili Kotmayer, Alexandra Balogh, Stefánia Gróf Ambrus, Gángó, Csaba Bödör, *[Genetic landscape and targeted therapy of chronic lymphocytic leukemia]*, 2021, Orvosképzés, 46(3), pp. 551-564 (Hungarian)

5. Lili Kotmayer, Krisztián Kállay, Csaba Bödör, *[Hereditary haematological malignancies]*, 2020, Magyar Onkológia, 64(1), pp. 43-55 (Hungarian)

6. Lili Kotmayer, Richárd Kiss, Attila Péter Király, Judit Csomor, Krisztián Kállay, Donát Alpár, Csaba Bödör, *[Screening for genomic copy number alterations in a family suffering from myelodysplastic syndrome using multiplex ligation-dependent probe amplification]*, 2018, Hematológia–Transzfuziológia, 51(4), 214-220 (Hungarian)

7. Oskar Marin-Bejar, Damia Romero-Moya, Javier Rodriguez-Ubreva, Maximiliano Distefano, Francesca Lessi, Paolo Aretini, Alessandro Liquori, Julio Castaño, Emilia Kozyra, Lili Kotmayer, Clara Bueno, José Cervera, José Carlos Rodriguez-Gallego, Josep F Nomdedeu, Laura Murillo-Sanjuán, Cristina Díaz de Heredia, Antonio Pérez-Martinez, Félix López-Cadenas, Carolina Martínez-Laperche, Nieves Dorado-Herrero, Francisco M Marco, Felipe Prósper, Pablo Menendez, David Valcárcel, Esteban Ballestar, Csaba Bödör, Anna Bigas, Albert Catalá, Marcin W Wlodarski, Alessandra Giorgetti, *Epigenome profiling reveals aberrant DNA methylation signature in GATA2 deficiency*, 2023, Haematologica, https://doi.org/10.3324/haematol.2022.282305

8. Ferenc Takács, Lili Kotmayer, Ágnes Czeti, Gábor Szalóki, Tamás László, Gábor Mikala, Ágnes Márk, András Masszi, Péter Farkas, Márk Plander, Júlia Weisinger, Judit Demeter, Sándor Fekete, László Szerafin, Beáta Margit Deák, Erika Szaleczky, Adrienn Sulák, Zita Borbényi, Gábor Barna, Revealing a Phenotypical Appearance of Ibrutinib Resistance Resistance in Patients With Chronic Lymphocytic Leukaemia by Flow Cytometry, 2023, Pathology Oncology Research, https://doi.org/10.3389/pore.2022.1610659

9. Gábor Bedics, Lili Kotmayer, Erik Zajta, Lajos László Hegyi, Edit Ágota Brückner, Hajnalka Rajnai, Lilla Reiniger, Csaba Bödör, Miklós Garami, Bálint Scheich, *Germline MUTYH mutations and high-grade gliomas: Novel evidence for a potential association*, 2022, Genes Chromosomes Cancer, 61(10), pp. 622-628

10. Noémi Jákob, Gábor Tamás Pintér, Lili Kotmayer, Péter Nagy, Csaba Bödör, Péter Barabás, Janka Bécser, György Szabó, Sándor Bogdán, *[Cherubism in three siblings]*, 2022, Orvosi Hetilap, 163(11), pp. 446-452 (Hungarian)

11. Zoltán Köllő, Lili Kotmayer, Barna Vásárhelyi, Gellért Balázs Karvaly, *[The role of modern therapeutic drug monitoring in the application of novel, small molecular weight, orally administered oncological drugs with specific molecular targets]*, 2021, Hematológia–Transzfuziológia, 54(4), pp. 174-183 (Hungarian)

12. Tamás László, Lili Kotmayer, Donát Alpár, Csaba Bödör, *[Venetoclax resistance in chronic lymphocytic leukemia]*, 2021, Hematológia–Transzfuziológia, 54(3), pp. 143-151 (Hungarian)

13. Ákos Nagy, Hajnalka Andrikovics, Béla Kajtár, Anikó Ujfalusi, Zsuzsanna László, Lili Kotmayer, Dóra Csabán, András Bors, Anett Makkos-Weisz, Emese Kapitány, Adrienn Sulák, Csaba Bödör, *[Analysis of the IGHV mutation status in the laboratories of the Molecular Diagnostic Workgroup of the Hungarian Society for Hematology and Transfusion]*, 2021, Hematológia–Transzfuziológia, 54(2), pp. 75-80 (Hungarian)

14. Ambrus Gángó, Richárd Kiss, Péter Farkas, Eid Hanna, Judit Demeter, Beáta Deák, Dóra Lévai, Lili Kotmayer, Donát Alpár, András Matolcsy, Csaba Bödör, Zoltán Mátrai, Botond Timár, *Morphologic and molecular analysis of Richter syndrome in chronic lymphocytic leukaemia patients treated with ibrutinib or venetoclax*, Pathology, 54(1), pp. 95-103

15. Ákos Nagy, Bence Bátai, Alexandra Balogh, Sarolta Illés, Gábor Mikala, Noémi Nagy, Laura Kiss, Lili Kotmayer, András Matolcsy, Donát Alpár, Tamás Masszi, András Masszi, Csaba Bödör, *Quantitative Analysis and Monitoring of EZH2 Mutations Using Liquid Biopsy in Follicular Lymphoma*, 2020, Genes, 11(7):785

16. Richárd Kiss, Gergő Papp, Szilvia Krizsán, Lili Kotmayer, Ambrus Gángó, Noémi Nagy, Bence Bátai, Zoltán Mátrai, Csaba Bödör, Donár Alpár, *[Screening for genomic copy number alterations in chronic lymphocytic leukemia using multiplex ligation-dependent probe amplification]*, 2018, Hematológia–Transzfuziológia, 51(1), pp. 31-40 (Hungarian)

DOI:10.14753/SE.2023.2857

X. ACKNOWLEDGEMENT

I would like to thank and acknowledge András Matolcsy, MD, PhD, Head of the Department of Pathology and Experimental Cancer Research at Semmelweis University, for allowing me to pursue PhD studies in this Institute.

I owe great thanks to my mentors and supervisors, Csaba Bödör, PhD, DSc, and Donát Alpár, PhD for years of kind support and advice, and for all the help I have received and still receive every day. They provide me with fantastic opportunities and freedom to participate in and carry out research projects. I value this trust greatly.

I wish to thank Richárd Kiss, MD, PhD for mentoring me in the early years and teaching me the basics of research. I am grateful for every time we discussed research ideas or presentations. His tireless support and constructive criticism helped me countless times to overcome challenges I faced doing science.

I would like to thank Tamás László, MD, my friend and colleague in CLL research, for working together over the past two years. His hardworking and inquisitive attitude towards research is exemplary and makes me grateful for all the experiences and discoveries we shared working on CLL.

I thank Gábor Bedics, MD, for his friendship and for his valuable contribution to resolving bioinformatic challenges I faced during my PhD. Although we only shared a few research projects, his insights helped me to learn and better understand science.

I wish to thank all the colleagues and members of the HCEMM-SE Molecular Oncohematology Research Group for creating a nourishing and inspiring work environment every day. I also owe thanks to every employee of the Institute for their support and eagerness to help.

I would like to thank my close friends, without whom this thesis would have never been completed. They were my pillars of support amidst the greatest storms.

Finally, I owe the greatest thanks to my family for their love and support over the years. I hope they know how their unconditional love helped through many challenges I faced during my PhD.

DOI:10.14753/SE.2023.2857

APPENDIX

Supplementary Tables related to the PhD thesis and Ethical License

Table 3. Patient characteristics in the analysed cohort treated with ibrutinib

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to ibrutinib therapy)	IGHV mutation status	TP53 status
P01	57	M	RFC, RCHOP, RB	1	C	NA	del(17p)	U	mutated
P02	56	F	CVP, R-CVP, RB	6	C	positive	12+	U	mutated
P03	48	F	FC, ofatumumab, RFC, RCHOP, RB	48	B	positive	12+	U	mutated
P04	56	M	Chl, RChl	10	A	NA	del(11q), del(13q)	U	wild type
P05	44	M	RFC	72	C	negative	del(13q), del(17p)	U	mutated
P06	37	F	RFC, RB	83	C	positive	del(13q), del(17p)	U	mutated
P07	63	F	RFC, RB	6	NA	positive	del(6q), del(13q), del(17p)	U	wild type
P08	37	F	FC, RFC, RB, RCVP	0	A	NA	negative	U	wild type
P09	61	F	RFC, CVP, RCHOP, CVP, Chl, B	1	B	positive	negative	U	mutated
P10	43	M	RFC, RB	15	B	NA	12+, del(13q), del(17p)	U	wild type
P11	26	M	FC, ofatumumab, RCHOP, RB, alemtuzumab	5	NA	NA	negative	U	wild type
P12	50	F	RFC, RB	32	C	NA	negative	U	wild type
P13	52	M	FC, RCVP, RFC, RB	6	C	positive	negative	U	mutated
P14	62	F	Chl, Cyc, RB	15	B	positive	12+, del(17p)	U	mutated
P15	43	F	FCM, RB, RCVP, RChl, RCHOP	59	C	positive	del(13q)	M	NA
P16	43	M	RFC, RB	1	C	positive	del(17p)	U	mutated
P17	73	M	FC, RB, RCVP, RHDMP	51	B	positive	del(13q)	M	wild type
P18	50	F	ChlFC, FC, RB	0	B	NA	del(13q)	U	mutated
P19	55	F	FC	11	A	NA	del(17p)	U	mutated
P20	37	M	RFC, duvelisib, RB	0	B	NA	negative	U	wild type
P21	59	M	RFC, RB	12	A	NA	del(11q)	U	wild type

DOI:10.14753/SE.2023.2857

Table 3. Patient characteristics in the analysed cohort treated with ibrutinib (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to ibrutinib therapy)	IGHV mutation status	TP53 status
P22	54	M	RFC, RB, RCVP	39	C	negative	negative	U	mutated
P23	61	F	RFC	84	A	NA	12+, del(17p)	U	mutated
P24	54	F	RFC, RB	39	C	NA	negative	U	mutated
P25	40	M	RCEOP, FC, RFC, RCVP	6	NA	NA	negative	U	mutated
P26	61	M	RCVP, RB	12	C	negative	del(11q)	U	wild type
P27	75	M	RChl, RFC, RB	6	C	NA	negative	U	wild type
P28	52	M	RB	90	A	positive	12+	U	wild type
P29	63	M	RFC, RB	2	NA	positive	del(11q)	U	NA
P30	60	M	RFC	1	C	positive	del(17p)	U	mutated
P31	54	M	RB	12	B	positive	negative	B	mutated
P32	65	M	RB, RChl, RCVP, RB, duvelisib	6	B	NA	negative	NA	wild type
P33	60	M	NA	72	A	NA	del(11q)	U	wild type
P34	57	M	RFC	3	B	NA	del(17p)	U	mutated
P35	52	M	RFC, alemtuzumab	11	A	NA	negative	U	mutated
P36	55	M	RFC	50	NA	NA	del(13q)	U	wild type
P37	55	M	RCVP, RFC, RB	11	C	NA	negative	NA	mutated
P38	42	M	RFC, RB	36	NA	NA	del(11q), del(13q)	U	wild type
P39	60	F	RCVP, RB, HD steroid & B & GCSF, RChl	13	NA	NA	del(17p)	U	mutated
P40	54	F	RFC, irradiation, alemtuzumab, RB	0	NA	positive	negative	U	wild type
P41	41	M	RFC	36	C	positive	del(11q), del(13q)	U	wild type
P42	59	F	RFC, RChl	20	C	positive	negative	U	wild type

DOI:10.14753/SE.2023.2857

Table 3. Patient characteristics in the analysed cohort treated with ibrutinib (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to ibrutinib therapy)	IGHV mutation status	TP53 status
P43	54	M	Chl, RFC	60	NA	NA	del(17p)	U	mutated
P44	62	M	RFC, R, B	0	C	positive	del(17p)	U	mutated
P45	61	M	FCR, RB	9	B	NA	del(11q), del(13q)	U	wild type
P46	73	M	RB & idelalisib	11	C	NA	del(17p)	U	mutated
P47	55	M	FC, RCHOP, RFC	12	A	negative	NA	U	mutated
P48	62	M	RFC	4	NA	negative	del(13q)	U	mutated
P49	62	F	RFC	57	C	NA	del(13q), del(17p)	M	mutated
P50	62	F	Chl, Flu, RFC , RB, RCVP	0	B	NA	negative	NA	NA
P51	60	F	RFC	0	C	NA	del(13q), del(17p)	U	wild type
P52	51	M	FCM, RB	36	B	positive	12+	U	mutated
P53	62	F	Chl, RFC	28	B	NA	NA	B	wild type
P54	42	M	FC, RB	0.5	C	negative	del(13q)	U	wild type
P55	61	M	Chl, RFC	48	B	NA	del(11q)	U	mutated
P56	52	M	FC, alemtuzumab	13	B	NA	negative	U	wild type
P57	58	F	RB	6	A	positive	negative	U	wild type
P58	63	M	Chl, RFC, RB	76	A	positive	del(13q)	U	wild type
P59	53	M	RFC, RB, Chl	10	B	positive	12+, del(13q)	U	mutated
P60	53	F	CHLB, FC, RFC	11	A	positive	negative	M	wild type
P61	55	M	RFC	20	C	positive	negative	U	wild type
P62	63	F	C, RChl	64	C	negative	negative	U	wild type
P63	59	M	RCVP, RCHOP	47	NA	positive	del(11q)	U	wild type
P64	71	F	RChl, RB	1	C	NA	12+	U	wild type

DOI:10.14753/SE.2023.2857

Table 3. Patient characteristics in the analysed cohort treated with ibrutinib (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to ibrutinib therapy)	IGHV mutation status	TP53 status
P65	54	F	RB	1	B	NA	del(11q)	U	wild type
P66	72	M	RFC	0	B	NA	del(17p)	U	mutated
P67	48	M	RFC	41	A	NA	negative	U	wild type
P68	65	M	RFC, RB	0	C	NA	12+	U	wild type
P69	61	F	Chl	48	NA	NA	negative	U	mutated
P70	66	M	RCVP	31	A	NA	del(13q), del(17p)	U	wild type
P71	55	F	RFC, RCHOP	14	NA	positive	del(11q)	U	wild type
P72	70	F	RCVP	95	NA	NA	del(17p)	U	mutated
P73	29	F	FC, RFC, RB	264	C	NA	del(17p)	M	mutated
P74	67	F	RFC, RB	9	C	positive	del(17p)	U	mutated
P75	70	F	alemtuzumab	1	A	positive	del(17p)	U	mutated
P76	77	F	obinutuzumab & Chl	51	B	NA	negative	U	NA
P77	52	M	RFC	5	A	NA	negative	U	wild type
P78	85	M	RB	28	B	NA	del(11q), del(13q)	U	NA
P79	40	M	RFC	72	A	NA	negative	M	wild type
P80	72	M	RB	10	A	NA	del(17p)	U	NA
P81	76	M	steroid, RB	0	A	NA	del(17p)	M	NA
P82	65	F	Chl, RB	36	A	NA	del(13q)	NA	wild type
P83	59	F	C, RFC, RC	31	B	NA	del(13q)	M	NA

12+: trisomy of chromosome 12; allo-SCT: allogeneic stem cell transplantation; Chl: chlorambucil; C: cyclophosphamide; del: deletion; F: female; FC: fludarabine, cyclophosphamide, FCM: fludarabine, cyclophosphamide, mitoxantrone; FISH: fluorescence *in situ* hybridization; Flu: fludarabine; HDMP: high dose methylprednisolone; M (Sex): male; M (IGHV mutation status): mutated; R: rituximab; RB: rituximab, bendamustin; RChl: rituximab, chlorambucile; RCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone; RCNOP: rituximab, cyclophosphamide, mitoxantrone, vincristine, prednisone; RCVP: rituximab, cyclophosphamide, vincristine, prednisolone; RFC: rituximab, fludarabine, cyclophosphamide; RM: ribomustin; TTFT: time to first treatment; U: unmutated

DOI:10.14753/SE.2023.2857

Table 4. Patient characteristics in the analysed cohort treated with venetoclax

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to venetoclax therapy)	IGHV mutation status	*TP53* status	Type of venetoclax therapy
P01	37	F	FC, RFC, RB, RCVP, ibrutinib	0	A	NA	negative	U	wild type	venetoclax monotherapy
P02	NA	M	ibrutinib	NA	B	NA	negative	NA	wild type	venetoclax monotherapy
P03	54	F	RC	5	NA	negative	del(13q)	B	wild type	venetoclax-rituximab combination
P04	50	M	RFC, ibrutinib	0	B	positive	negative	U	wild type	venetoclax-rituximab combination
P05	50	M	RFC	75	NA	negative	negative	U	wild type	venetoclax monotherapy
P06	63	M	RFC, RB, ibrutinib	2	B	positive	del(11q)	U	wild type	venetoclax monotherapy
P07	33	F	RFC, RB, idelalisib, irradiation, ibrutinib	0	NA	NA	NA	NA	NA	venetoclax monotherapy
P08	54	F	RFC, irradiation, alemtuzumab, RB, ibrutinib	0	NA	positive	negative	U	wild type	venetoclax monotherapy
P09	35	F	FC, alemtuzumab, RB, idelalisib	2	NA	positive	negative	U	positive	venetoclax-rituximab combination
P10	54	M	RB, ibrutinib	12	B	positive	negative	B	positive	venetoclax monotherapy
P11	46	M	RFC, ibrutinib	NA	NA	NA	del(11q), +12, del(13q), del(17p), i(17q)	U	positive	venetoclax monotherapy
P12	46	M	idelalisib, ibrutinib	NA	NA	dim	NA	NA	NA	venetoclax monotherapy

DOI:10.14753/SE.2023.2857

Table 4. Patient characteristics in the analysed cohort treated with venetoclax (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to venetoclax therapy)	IGHV mutation status	*TP53* status	Type of venetoclax therapy
P13	49	M	RFC	26	NA	negative	del(11q)	U	positive	venetoclax monotherapy
P14	70	F	RB	19	NA	positive	negative	U	positive	venetoclax-rituximab combination
P15	47	F	RFC, RC	8	C	positive	negative	NA	NA	venetoclax monotherapy
P16	50	F	RFC, RB, ibrutinib	32	C	NA	negative	U	wild type	venetoclax monotherapy
P17	37	F	RFC, RB, ibrutinib	83	C	positive	del(13q), del(17p)	U	positive	venetoclax monotherapy
P18	73	M	obinutuzumab-Chl	62	NA	positive	del(11q)	U	wild type	venetoclax-rituximab combination
P19	55	M	RFC, RB	12	NA	positive	del(11q), +12, del(13q), del(17p)	U	wild type	venetoclax monotherapy
P20	75	M	RChl, RFC, RB, ibrutinib	6	C	NA	negative	U	wild type	venetoclax monotherapy
P21	37	M	RFC, IPI-145, RB, ibrutinib	0	B	NA	negative	U	wild type	venetoclax monotherapy
P22	66	F	Chl, cyclophosphamide, ofatumumab, duvelisib, RB, ibrutinib	96	B	positive	del(11q)	U	wild type	venetoclax monotherapy
P23	50	M	F, cyclophosphamide, RB, RFC	0	NA	NA	negative	U	wild type	venetoclax-rituximab combination

DOI:10.14753/SE.2023.2857

Table 4. Patient characteristics in the analysed cohort treated with venetoclax (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to venetoclax therapy)	IGHV mutation status	*TP53* status	Type of venetoclax therapy
P24	57	F	RFC	33	A	positive	negative	M	positive	venetoclax monotherapy
P25	56	M	Chl, RChl, ibrutinib	10	A	NA	del(11q), del(13q)	U	wild type	venetoclax monotherapy
P26	57	M	RFC, RCHOP, RB, ibrutinib	1	C	NA	del(17p)	U	positive	venetoclax monotherapy
P27	71	M	RCVP, RFC lite, RB	10	A	positive	del(13q), t(14;?)	U	wild type	venetoclax monotherapy
P28	42	M	RFC, IBR	4	NA	negative	del(11q)	U	wild type	venetoclax monotherapy
P29	60	F	cyclophosphamide, Chl	60	NA	NA	negative	U	wild type	venetoclax-rituximab combination
P30	63	F	RFC, ibrutinib	15	C	NA	+12, del(13q), del(17q)	NA	NA	venetoclax monotherapy
P31	73	M	RChl, RB	0	B	positive	del(13q), del(17p)	NA	positive	venetoclax monotherapy
P32	40	M	RCEOP, FC, RFC, RCVP, ibrutinib	6	NA	positive	del(11q)	U	positive	venetoclax monotherapy
P33	49	M	acalabrutinib	9	C	positive	+12	U	wild type	venetoclax monotherapy
P34	63	M	Chl, RFC, RB, ibrutinib	76	A	positive	del(13q)	U	wild type	venetoclax monotherapy
P35	44	M	RFC, ibrutinib	72	C	negative	del(13q), del(17p)	U	positive	venetoclax monotherapy

DOI:10.14753/SE.2023.2857

Table 4. Patient characteristics in the analysed cohort treated with venetoclax (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to venetoclax therapy)	IGHV mutation status	*TP53* status	Type of venetoclax therapy
P36	59	M	RFC	1	NA	positive	del(11q)	U	wild type	venetoclax-rituximab combination
P37	56	F	CVP, RCVP, RB, ibrutinib	6	C	positive	+12	U	positive	venetoclax monotherapy
P38	60	F	RCVP, RB, high dose steroid + B, RChl, ibrutinib	12	NA	NA	del(17p)	U	positive	venetoclax monotherapy
P39	38	M	RFC, FC	54	B	negative	del(11q)	M	wild type	venetoclax monotherapy
P40	62	M	RFC, R + idelalisib, RB	0	NA	NA	NA	U	wild type	venetoclax monotherapy
P41	37	F	RFC, alemtuzumab, RB, RCHOP, ibrutinib	9	B	positive	del(17p)	NA	positive	venetoclax monotherapy
P42	59	M	RFC, acalabrutinib	1	C	NA	del(11q)	U	wild type	venetoclax monotherapy
P43	48	F	FC, ofatumumab, RFC, RCHOP, RB, ibrutinib	48	B	positive	+12, t(14;?)	U	positive	venetoclax monotherapy
P44	63	M	RB	2	NA	NA	del(11q)	U	wild type	venetoclax-rituximab combination
P45	70	F	ibrutinib	6	NA	NA	del(13q), del(17p)	NA	NA	venetoclax monotherapy
P46	54	M	FC, RB	12	A	negative	negative	M	wild type	venetoclax monotherapy
P47	69	M	RFC	18	A	NA	negative	NA	NA	venetoclax monotherapy

DOI:10.14753/SE.2023.2857

Table 4. Patient characteristics in the analysed cohort treated with venetoclax (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to venetoclax therapy)	IGHV mutation status	*TP53* status	Type of venetoclax therapy
P48	54	M	RChl, RB, alemtuzumab, Chl, RCHOP, ibrutinib	0	C	positive	negative	NA	wild type	venetoclax monotherapy
P49	65	M	lenalinomide, RChl	NA	NA	NA	del(17p)	NA	NA	venetoclax monotherapy
P50	43	F	FCM, RB, RCVP, RChl, RCHOP, ibrutinib	59	C	positive	del(13q)	M	positive	venetoclax monotherapy
P51	59	F	RFC, RChl, ibrutinib	20	C	NA	negative	U	wild type	venetoclax monotherapy
P52	52	M	RB, ibrutinib	90	A	positive	+12	U	wild type	venetoclax monotherapy
P53	64	F	RB, ibrutinib	3	C	negative	del(17p)	U	positive	venetoclax monotherapy
P54	52	M	RFC, ibrutinib	84	NA	positive	negative	U	NA	venetoclax monotherapy
P55	58	M	RFC	1	B	positive	del(17p)	NA	positive	venetoclax monotherapy
P56	51	M	RFC, RB	12	NA	positive	negative	U	positive	venetoclax-rituximab combination
P57	66	M	RFC, B	4	B	positive	negative	NA	NA	venetoclax monotherapy
P58	41	M	RFC, ibrutinib	36	C	positive	del(11q), del(13q)	U	wild type	venetoclax monotherapy
P59	46	M	alemtuzumab, allo-SCT, ibrutinib	61	B	positive	del(17p)	B	NA	venetoclax monotherapy

DOI:10.14753/SE.2023.2857

Table 4. Patient characteristics in the analysed cohort treated with venetoclax (continued)

Patient ID	Age at diagnosis (yrs)	Sex	Prior therapies	TTFT (months)	Binet stage	CD38 expression	Cytogenetics (prior to venetoclax therapy)	IGHV mutation status	*TP53* status	Type of venetoclax therapy
P60	55	M	RFC, ibrutinib	20	C	negative	negative	U	wild type	venetoclax monotherapy
P61	71	M	RB	75	C	NA	del(13q)	M	positive	venetoclax-rituximab combination
P62	53	M	RFC	47	NA	positive	+12, t(14:19)	U	wild type	venetoclax-rituximab combination
P63	60	M	RB	26	C	NA	NA	NA	NA	venetoclax-rituximab combination
P64	69	M	RChl, RFC, RB, ibrutinib	21	NA	negative	negative	NA	positive	venetoclax monotherapy
P65	52	M	RB	8	NA	NA	del(11q), del(13q)	U	wild type	venetoclax-rituximab combination
P66	63	M	R + idelalisib	58	NA	NA	negative	U	wild type	venetoclax-rituximab combination
P67	74	M	ibrutinib	25	NA	NA	del(17p)	M	positive	venetoclax-rituximab combination

* venetoclax therapy was discontinued due to disease progression or toxicity

** termination of venetoclax therapy due to fixed-duration treatment or sustained deep remission

+12: trisomy of chromosome 12; allo-SCT: allogeneic stem cell transplantation; Chl: chlorambucil; C: cyclophosphamide; del: deletion; F (Sex): female; F (Prior therapies): fludarabine; FC: fludarabine, cyclophosphamide, FCM: fludarabine, cyclophosphamide, mitoxantrone; FISH: fluorescence *in situ* hybridization; i: isochromosome; M (Sex): male; M (IGHV mutation status): mutated; R: rituximab; RB: rituximab, bendamustin; RChl: rituximab, chlorambucil; RCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone; RCVP: rituximab, cyclophosphamide, vincristine, prednisolone; RFC: rituximab, fludarabine, cyclophosphamide; t: translocation; TTFT: time to first treatment; U: unmutated

DOI:10.14753/SE.2023.2857

Supplementary Table 3. Variants detected by ultra-deep next generation sequencing in patients progressing on ibrutinib but not harboring *BTK* p.Cys481Ser

Patient ID	Chromosome	Position	Gene Name	Gene ID	HGVS.c	HGVS.p	Variant ID	ClinVar significance
P46	chr17	7579395	*TP53*	ENSG00000141510	c.288_291delTGTC	p.Val97fs	previously not reported	-
P48	chr17	7576897	*TP53*	ENSG00000141510	c.949C>T	p.Gln317*	COSM10786; COSM165085; COSM1709728; COSM3388166; rs764735889	pathogenic
P52	chr17	7577509	*TP53*	ENSG00000141510	c.772G>A		COSM10988; COSM1172461; COSM1172462; COSM1679494; rs121912652	pathogenic
P72	chr17	7577105	*TP53*	ENSG00000141510	c.833C>G	p.Pro278Arg	COSM10887;COSM1646811; COSM300205	-
P75	chr16	81927328	*PLCG2*	ENSG00000197943	c.1001A>G	p.Asp334Gly	rs372563994	uncertain significance
P75	chr17	7579528	*TP53*	ENSG00000141510	c.159G>A	p.Trp53*	COSM1324762; COSM1324763; COSM44488	-

DOI:10.14753/SE.2023.2857

DOI:10.14753/SE.2023.2857

Supplementary Table 4. Variants detected by ultra-deep next generation sequencing in the samples of patients progressing on venetoclax

Patient ID	Chromosome	Position	Gene Name	Gene ID	HGVS.c	HGVS.p	Variant COSMIC ID	ClinVar significance
P10	chr17	7577574	*TP53*	ENSG00000141510	c.707A>G	p.Tyr236Cys	COSM10731	uncertain (pathogenic)
P10	chr16	81962185	*PLCG2*	ENSG00000197943	c.2537G>T	p.Gly846Val		not reported
P10	chr16	81969909	*PLCG2*	ENSG00000197943	c.2978A>G	p.Asp993Gly		not reported
P10	chrX	100611164	*BTK*	ENSG00000010671	c.1442G>C	p.Cys481Ser		not reported
P13	chr17	7577112	*TP53*	ENSG00000141510	c.826G>C	p.Ala276Pro	COSM308313	pathogenic
P31	chr16	81891928	*PLCG2*	ENSG00000197943	c.398C>T	p.Ala133Val		conflicting
P31	chr17	7577114	*TP53*	ENSG00000141510	c.824G>T	p.Cys275Phe	COSM10701	pathogenic
P31	chr17	7577538	*TP53*	ENSG00000141510	c.743G>A	p.Arg248Gln	COSM10662	pathogenic
P31	chr17	7577539	*TP53*	ENSG00000141510	c.742C>T	p.Arg248Trp	COSM10656	pathogenic
P31	chr17	7578271	*TP53*	ENSG00000141510	c.578A>G	p.His193Arg	COSM10742	pathogenic
P31	chr17	7578394	*TP53*	ENSG00000141510	c.536A>G	p.His179Arg	COSM10889	pathogenic
P31	chr18	60985433	*BCL2*	ENSG00000171791	c.467T>A	p.Val156Asp		not reported
P31	chr18	60985562	*BCL2*	ENSG00000171791	c.338C>G	p.Ala113Gly	COSM3357021	not reported

DOI:10.14753/SE.2023.2857

Supplementary Table 4. Variants detected by ultra-deep next generation sequencing in the samples of patients progressing on venetoclax (continued)

Patient ID	Chromosome	Position	Gene Name	Gene ID	HGVS.c	HGVS.p	Variant COSMIC ID	ClinVar significance
P31	chr18	60985591	*BCL2*	ENSG00000171791	c.309C>G	p.Asp103Glu		not reported
P31	chr18	60985593	*BCL2*	ENSG00000171791	c.307G>T	p.Asp103Tyr		not reported
P31	chr18	60985598	*BCL2*	ENSG00000171791	c.302G>T	p.Gly101Val		not reported
P38	chr17	7578398	*TP53*	ENSG00000141510	c.532C>G	p.His178Asp	COSM44901	pathogenic
P38	chrX	100611185	*BTK*	ENSG00000010671	c.1421C>T	p.Tyr474Ile		not reported
P40	chr17	7577112	*TP53*	ENSG00000141510	c.826G>C	p.Ala276Pro	COSM308313	pathogenic
P40	chr18	60985562	*BCL2*	ENSG00000171791	c.338C>G	p.Ala113Gly	COSM3357021	not reported
P40	chr18	60985591	*BCL2*	ENSG00000171791	c.309C>A	p.Asp103Glu		not reported
P40	chr18	60985593	*BCL2*	ENSG00000171791	c.307G>T	p.Asp103Tyr		not reported
P43	chrX	100611164	*BTK*	ENSG00000010671	c.1442G>A	p.Cys481Tyr		not reported
P55	chr17	7578552	*TP53*	ENSG00000141510	c.378C>A	p.Tyr126*	COSM10888	pathogenic
P08	chrX	100611164	*BTK*	ENSG00000010671	c.1442G>C	p.Cys481Ser		not reported
P11	chr17	7578263	*TP53*	ENSG00000141510	c.586C>T	p.Arg196*	COSM10705	pathogenic

DOI:10.14753/SE.2023.2857

Supplementary Table 4. Variants detected by ultra-deep next generation sequencing in the samples of patients progressing on venetoclax (continued)

Patient ID	Chromosome	Position	Gene Name	Gene ID	HGVS.c	HGVS.p	Variant COSMIC ID	ClinVar significance
P25	chr16	81888131	*PLCG2*	ENSG00000197943	c.276A>C	p.Glu92Asp		not reported
P25	chr17	7576570	*TP53*	ENSG00000141510	c.1008A>C	p.Leu336Phe	COSM1610827	pathogenic
P25	chrX	100611164	*BTK*	ENSG00000010671	c.1442G>C	p.Cys481Tyr		not reported
P53	chr17	7577538	*TP53*	ENSG00000141510	c.743G>A	p.Arg248Gln	COSM10662	pathogenic

DOI:10.14753/SE.2023.2857

Egészségügyi Tudományos Tanács
Tudományos és Kutatásetikai Bizottság (ETT TUKEB)
Levelezési cím: H-1051 Budapest, Széchenyi István tér 7-8.
Székhely: Budapest 1054 Alkotmány u. 25.

Ügyiratszám: **IV/5495- 3 /2021/EKU**
Ügyintéző neve: **Dr. Kardon Tamás titkár**
Elérhetősége: **tukeb@emmi.gov.hu**
Telefon: **+(36) 1 795-1197**

Tárgy: Engedélyező határozat

Kutatóhely neve: *Semmelweis Egyetem ÁOK I.sz. Patológiai és Kísérleti Rákkutató Intézet*
Kutatóhely címe:
Budapest
Üllői út 26.
1085

Kutatásvezető: Dr. Bödör Csaba *részére*

HATÁROZAT

A(z) Semmelweis Egyetem, HCEMM, és a Nemzeti Kutatási, Fejlesztési És Innovációs Hivatal, mint megbízó (1085 Budapest Üllői út 26.) képviseletében Kővári Krisztina, Takó Tamás (1085 Budapest Üllői út 26.) (továbbiakban: Kérelmező) "B-sejtes non-Hodgkin limfómák térbeli heterogenitásának és klonális evolúciójának vizsgálata" című, beavatkozással nem járó vizsgálat engedélyezése iránt kérelmet nyújtott be az Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottságához (az ETT TUKEB-hez).

Az ETT TUKEB, mint **elsőfokú** hatóság, a vizsgálat engedélyezése iránti kérelmet megvizsgálta és a következő, testületi véleményen alapuló döntést hozta:

Az ETT TUKEB a benyújtott kérelem szerinti, beavatkozással nem járó vizsgálatra

a szakmai-etikai engedélyt megadja.

Az eljárás során eljárási költség nem merült fel tekintettel arra, hogy a kérelmezett vizsgálat nem kereskedelmi vizsgálat.

A Bizottság döntése ellen a közlést követő 15 napon belül van helye fellebbezésnek az ETT Elnökségéhez. A fellebbezést az ETT TUKEB-hez kell benyújtani.

A fellebbezési eljárás illeték- és díjmentes.

INDOKOLÁS

A Kérelmező "B-sejtes non-Hodgkin limfómák térbeli heterogenitásának és klonális evolúciójának vizsgálata" című, beavatkozással nem járó vizsgálat engedélyezése iránt kérelmet nyújtott be ETT TUKEB-hez, ami 2021. június 8-én érkezett meg a Bizottsághoz.

Az eljárás megindult és az ETT TUKEB az általános közigazgatási rendtartásról szóló 2016. évi CL. törvény (továbbiakban: *Ákr.*) 43.§ (1) bekezdésében foglalt intézkedéseket mellőzte, és tekintettel arra, hogy a teljes eljárás feltételei fennállnak, a Bizottság a kérelmet az *Ákr* 43 §-a alapján teljes eljárásban bírálta el. Az ETT TUKEB az *Ákr* 43 § (2) bekezdése alapján 2021. június 16-án erről és az *Ákr* 43 § (2) bekezdésében meghatározott egyéb tényekről tájékoztatta a Kérelmezőt.

Az eljárás során az ETT TUKEB megvizsgálta és megtárgyalta a kérelmet és a csatolt dokumentumokat.

A tervezett, beavatkozással nem járó vizsgálat azonosító adatai:

 DOI:10.14753/SE.2023.2857

A vizsgálat címe:
"B-sejtes non-Hodgkin limfómák térbeli heterogenitásának és klonális evolúciójának vizsgálata"

Kutatásvezető, aki az egész vizsgálatot vezeti: Dr. Bödör Csaba
Kutatásvezető munkahely: Semmelweis Egyetem ÁOK I.sz. Patológiai és Kísérleti Rákkutató Intézet

Megbízó neve és címe:
Semmelweis Egyetem, HCEMM, és a Nemzeti Kutatási, Fejlesztési És Innovációs Hivatal
1085 Budapest, Üllői út 26.

Megbízó képviselőjének a neve és címe:
Kővári Krisztina, Takó Tamás
1085 Budapest, Üllői út 26.

A vizsgálat tervezett időtartama: 2021.09.01 - 2025.08.31

A vizsgálatban részt vevő vizsgálóhelyek felsorolása, valamint az adott vizsgálóhelyen a vizsgálat vezetője:

- Debreceni Egyetem, II.sz. Belgyógyászati Klinika, Debrecen
- Dél-Pesti Centrumkórház – Országos Hematológiai és Infektológiai Intézet, Hematológiai és Őssejttranszplantációs Osztály, Budapest
- Fejér Megyei Szent György Egyetemi Oktató Kórház, III. Belgyógyászati Osztály - Hematológiai Osztály, Székesfehérvár
- Országos Onkológiai Intézet, Gyógyszerterápiás Központ Hematológia és Lymphoma Osztály, Budapest
- Jász-Nagykun-Szolnok Megyei Hetényi Géza Kórház-Rendelőintézet, I. Belgyógyászati Osztály,
- Magyar Honvédség Egészségügyi Központ, I.sz. Belgyógyászati Osztály, Budapest
- Markhot Ferenc Oktatókórház és Rendelőintézet, Eger
- Markusovszky Egyetemi Oktatókórház, Haematológiai és Haemosztazeológiai Osztály, Szombathely
- Pécsi Tudományegyetem, I.sz. Belgyógyászati Klinika, Hematológiai Tanszék, Pécs
- Pécsi Tudományegyetem, Szentágothai János Kutatóközpont, Bioinformatikai Kutatócsoport, Pécs
- Semmelweis Egyetem, Belgyógyászati és Hematológiai Klinika, Hematológiai Osztály, Budapest
- Semmelweis Egyetem, Belgyógyászati és Onkológiai Klinika, Hematológiai Osztály, Budapest
- Semmelweis Egyetem, I.sz. Patológiai és Kísérleti Rákkutató Intézet, Budapest
- Somogy Megyei Kaposi Mór Oktató Kórház, Hematológiai Osztály, Kaposvár
- Szabolcs-Szatmár-Bereg Megyei Kórházak és Egyetemi Oktatókórház, Hematológiai Osztály
- Szegedi Tudományegyetem, II.sz. Belgyógyászati Klinika, Szeged

Az ETT TUKEB a kutatási engedély iránti kérelemről *az emberen végzett orvostudományi kutatások, az emberi felhasználásra kerülő vizsgálati készítmények klinikai vizsgálata, valamint az emberen történő alkalmazásra szolgáló, klinikai vizsgálatra szánt orvostechnikai eszközök klinikai vizsgálata engedélyezési eljárásának szabályairól szóló 235/2009. (X. 20.) Korm. rendelet* (a továbbiakban: *235/2009. Korm. rendelet*) 18. § (2) bekezdése alapján a következőket állapította meg:

a) A beadott kérelem tárgyául szolgáló vizsgálat valóban beavatkozással nem járó vizsgálat-e?
Igen

b1) A tervezett vizsgálat érdemi, szakmai tudományos kérdésfelvetéseket tartalmaz-e?
Igen

b2) A tervezett vizsgálat módszerei alkalmasak-e az érdemi, szakmai tudományos kérdésfelvetések megválaszolására?
Igen

c1) A betegtájékoztató és a beleegyező nyilatkozat tervezett szövege megfelel-e az emberen végzett orvostudományi kutatásokról szóló miniszteri rendeletben foglaltaknak?
Igen

DOI:10.14753/SE.2023.2857

c2) A toborzás tervezett szövege megfelel-e az emberen végzett orvostudományi kutatásokról szóló miniszteri rendeletben foglaltaknak?
Igen

Mindezek alapján az ETT TUKEB a rendelkező résznek megfelelően határozott, és engedélyezte a kutatási engedély iránti kérelemben megjelölt beavatkozással nem járó vizsgálatot.

Felhívjuk a Kérelmező figyelmét arra a jogszabályi kötelezettségére, mely szerint a beavatkozással nem járó vizsgálat befejezését követő kilencven napon belül értesítenie kell az ETT TUKEB-et a vizsgálat befejezéséről, a bevont betegek számáról, illetve köréről, továbbá a vizsgálat befejezését követő száznyolcvan napon belül értesítenie kell az ETT TUKEB-et a vizsgálat célkitűzésére adott válaszról. (Ezt az előírást *az emberen végzett orvostudományi kutatásokról szóló 23/2002. (V. 9.) EüM rendelet* (továbbiakban: *23/2002. (V. 9.) EüM rendelet*) 20/O. § tartalmazza.)

Kérjük, amennyiben a beavatkozással nem járó vizsgálat nem kezdődik el, vagy idő előtt lezárásra kerül, akkor erről - az indokok felsorolásával - e-mailen és levélben is tájékoztassa az ETT TUKEB-et. (Ezt a *235/2009. Korm. rendelet* 21. § (3) bekezdése írja elő.)

Az ETT TUKEB eljárása és határozata elsősorban *az egészségügyről szóló 1997. évi CLIV. törvény* (továbbiakban: *Eütv.*) 164/A. § (1) és (2).bekezdésein, a *235/2009. (X. 20.) Korm. rendelet* 17/A. § (1) - (4), a 18. § (1) és (2) bekezdésein, valamint a az *Ákr.* 80. § (1) bekezdésén és az *Ákr.* 81. § (1) és (4) bekezdésein alapul.

A kutatásengedélyezési eljárásokban az *Eütv.* 164/B. § kimondja "Az orvostudományi kutatás, valamint a 164/A. § szerinti beavatkozással nem járó vizsgálat engedélyezési eljárásáért - az egészségügyért felelős miniszternek az adópolitikáért felelős miniszterrel egyetértésben kiadott rendeletében meghatározott - igazgatási szolgáltatási díjat kell fizetni."
A kérelemben foglaltak alapján az ETT TUKEB megállapította, hogy a tervezett kutatás nem kereskedelmi vizsgálat a *23/2002. EüM. rend.* 20/B. § f) pontja alapján, ezért a Kérelmezőnek eljárási költség, és így igazgatási szolgáltatási díjfizetési kötelezettsége sem keletkezett a *23/2002. EüM. rend.* 15. §-a és 20/R. § (1) bekezdése szerint.

A Bizottság hatásköre és illetékessége az *Eütv.* 164/A. § (2) bekezdésén, valamint *235/2009. Korm. rend.* 16. § a) pontján, a 17. § (1) bek. a) pontja ab) alpontján alapul.

A fellebbezés lehetőségét az *Ákr.* 116. § (1) bekezdésének megfelelően az *Eütv.* 164/A. § (2) bekezdése mondja ki, mely szerint "A (3) és (5) bekezdésben nem említett beavatkozással nem járó vizsgálat esetében a szakmai-etikai engedélyről az emberen végzett orvostudományi kutatásokról szóló kormányrendelet szerinti kutatás-etikai bizottság a kérelem megérkezését követő naptól számított negyvenöt napon belül dönt. A döntés ellen fellebbezésnek van helye, a másodfokú eljárást az ETT elnöksége folytatja le."

A fellebbezési eljárás illetékmentességét *az illetékekről szóló 1990. évi XCIII. Törvény* 67. § (3)-(5) bekezdései alapján a *23/2002. EüM. rend.* 15. §-a és 20/R. § (1) bekezdése mondja ki, tekintettel arra, hogy a kérelmezett kutatás nem kereskedelmi vizsgálat a *23/2002. EüM. rend.* 20/B. § f) pontja alapján.

A fellebbezés előterjesztésére az *Ákr.* 118. § (3) bekezdése vonatkozik.

Budapest, 2021. július 6.

P.H.

Prof. Dr. Schaff Zsuzsa ETT TUKEB
elnök nevében kiadmányozza:

Dr. Kardon Tamás ETT TUKEB titkár

Kapják:
1./ Kutatásvezető
2./ Intézetvezető
3./ Intézményvezető
4./ Irattár

Ügyiratszám: IV/5495- 3 /2021/EKU